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*Blood Pressure, Report of Joint Committee on Mortality of The Association of Life Insurance Medical Directors and The Actuarial Society of America, New York, 1925, pp. 10, 52.

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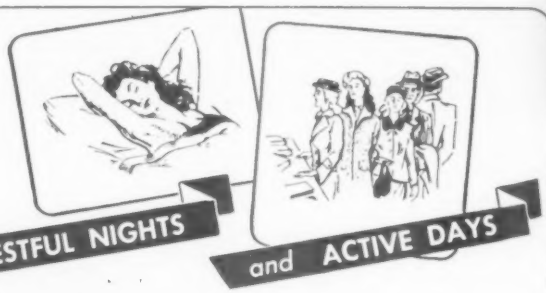

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1. Schwartz, I. R.; Lehman, E.; Ostrove, R., and Seibel, J. M.: A Clinical Evaluation of a New Anticholinergic Drug, Pro-Banthine, to be published.

2. Ruffin, J. M.; Baylin, G. J.; Legerton, C. W., Jr., and Texter, E. C., Jr.: Mechanism of Pain in Peptic Ulcer, Gastroenterology *23*:252 (Feb.) 1953.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

DECEMBER, 1953

ORIGINAL ARTICLES

THE USE OF CORRECTIVES IN THE PREVENTION OF BARBITURATE INTOXICATION*

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The term "corrective" in modern therapeutics has become obsolete largely because it was thought to be a concept of empirical rather than of rational therapeutics. This term should be redefined, particularly in view of the introduction of potent, effective, and potentially toxic therapeutic agents that produce many undesirable side actions which may be prevented by the simultaneous administration of another drug. A corrective is a drug that protects the patient against the undesirable side actions and/or the overdosage of another drug without interfering with the intended therapeutic effect of the latter. The present paper deals with the use of correctives in the prevention of acute barbiturate poisoning.

There are 3 possible ways to approach the problem of the prevention of acute barbiturate poisoning: 1) Corrective A: One would be to interfere with absorption from the gastrointestinal tract by utilizing agents that produce vomiting or diarrhea, or to increase the rate of destruction of these compounds in the lumen of the gastrointestinal tract. 2) Corrective B: The second would be the employment of antagonists that would enhance the elimination or metabolism of the barbiturates after they have been absorbed. 3) Corrective C: The third approach would consist of the use of physiological antidotes which either stimulate at the same sites which barbiturates depress or act as competitive biological antagonists for identical enzyme centers. Corrective drugs, depending on the site and method of action, must fulfill certain criteria in order to be clinically valuable in the prevention of barbiturate poisoning.

1. corrective a. Agents designated as "Corrective A" must possess the following characteristics: 1) They should not interfere with the intended therapeutic effects of the barbiturates, that is, in small doses they should not cause emesis or catharsis or chemically react with the barbiturates. 2) The ingestion of large doses of their combinations

*The Tetrobrarb combinations were supplied by Strong Cobb & Company, Inc., Cleveland, Ohio.

with the barbiturates must produce emesis or diarrhea by local irritation. 3) They must not produce severe gastroenteritis or colitis. 4) They may produce chemical reaction with double decomposition of the barbiturates in the gastrointestinal tract. 5) They should be non-absorbable because by their very nature they are irritating or toxic drugs. If they are absorbed in trace amounts, they should produce no chronic toxicity (cumulative effects), either by themselves or in the presence of barbiturates. (6) Finally, agents of this nature should act promptly so that absorbed barbiturates will not depress the vomiting center or gastrointestinal motility.

Several drugs of this category have already been tried in the prevention of barbiturate poisoning, but they have not been recommended for clinical use because they failed to meet some of the above criteria. Radomski *et al.*[21] showed that ipecac did not produce prompt emesis in the presence of large doses of barbiturates. Upon repeated administration, it was absorbed and produced liver damage and bone marrow hypoplasia in dogs. Zinc salts (Miskimon and Miskimon[18]), in combination with large doses of barbiturates, produce prompt emesis but because of their chronic toxicity they were never adopted clinically. Our unpublished data indicate that the simultaneous oral administration to dogs of magnesium sulfate or sodium phosphate with pentobarbital sodium in a ratio of 20 to 1 did not interfere with the onset of barbiturate anesthesia although in the majority of the cases copious diarrhea ensued. Hatcher[10] has shown that some but not all barbiturates are oxidized by $KMnO_4$. The utilization of oxidizing agents is not feasible because therapeutic doses may be destroyed by such powerful agents, and also the absorption of either excess of oxidizing agent or of the oxidation products may give rise to undesirable, if not dangerous, effects. Although it would not be impossible to manufacture tablets or capsules containing a chemical antidote or an adsorbent and a barbiturate in separate compartments, the above objections would still hold.

Finally, the incorporation of barbiturates in a non-digestible substance (chicle) might be considered. Chicle containing barbiturate would have to be chewed until all of its barbiturate content is liberated into the oral cavity. To commit suicide or fall victim to "automatic ingestion" of barbiturates, the individual must continue chewing, and this may be impossible because of the soporific effect produced by the absorbed, non-fatal dose of barbiturate. Chicle or similar substances are indigestible so that if swallowed, at the most only an insignificant amount of the barbiturate would be absorbed. To our knowledge, this method of barbiturate administration has not been attempted, and it may prove to be impractical.

2. CORRECTIVE B. Drugs that enhance the elimination or increase the metabolic destruction of barbiturates might also be incorporated into pharmaceutical preparations of a barbiturate, provided they fulfill the following criteria: 1) They should be absorbed simultaneously or almost simultaneously with the barbiturate. 2) They should not produce nausea, vomiting, or diarrhea. 3) They should not nullify the effects of therapeutic doses of barbiturates; however, they should protect against larger doses of barbiturates. 4) They should not produce untoward, acute or chronic effects of their own.

Hitherto, no drugs have been discovered that can enhance either the elimination or the metabolic destruction of barbiturates. It is true that massive intravenous saline or glucose infusions[6] or dialysis by means of an

artificial kidney[15,20] may increase the elimination of barbiturates, but such procedures hardly come within our purview. It is conceivable that an agent may be discovered which will selectively inhibit the tubular reabsorption of barbiturates and thus increase their renal elimination. Drugs are already known that inhibit carbonic anhydrase and thus favor the elimination of sodium and potassium[3]. Similarly, phlorizin inhibits phosphorylating enzymes within the renal tubules enhancing the elimination of glucose[17]. Probenicid (Benemid) produces a highly selective suppression of the tubular reabsorption of urates[9]. Although Blau[4] has stated that hyperthyroid rats recovered earlier than controls from sodium amytal anesthesia, for obvious reasons thyroid preparations could not be used as barbiturate correctives. It is possible that less toxic and more quickly acting agents may be found. At any rate, suitable drugs accelerating the elimination or metabolic destruction of barbiturates are not available at the present time.

3. CORRECTIVE C. Physiological antagonists to barbiturates have long been used but only for counteracting an already existing coma and not for its prevention. Combinations of physiological antidotes with pharmaceutical preparations containing barbiturates must fulfill the following desiderata: 1) The antagonist should be absorbed at approximately the same rate as the barbiturate, and following absorption their onset of action should be about the same. 2) They should not be irritating to the mucosa of the alimentary tract. 3) Their action should be such that when administered with barbiturates in mixtures of fixed proportions they should not interfere with the intended therapeutic effects of barbiturates, but in case of overdosage they should lighten the coma and prolong life. 4) The duration of action of the antagonist on the central nervous system should not be wholly out of proportion with the duration of action of the barbiturate. It is not deemed necessary that the duration of action of these 2 drugs should be exactly the same; all that is necessary is that the patient should be less depressed and more amenable to treatment. 5) The antagonist should cause neither acute nor chronic toxicity by itself or in combination with a barbiturate. 6) The corrective should not be habit forming or an addiction liability.

From a practical viewpoint it is also desirable that all suitable correctives should be readily available in sufficient quantities for manufacturing use, and their price should be within the reach of the general public.

This report deals more specifically with the employment of barbiturate correctives of the central analeptic type. Among the many central analeptics used, picrotoxin and pentylenetetrazol have been found experimentally and clinically to be the most effective in the treatment of acute barbiturate intoxication. Experimentally coriamyrtin was also found to be very effective. This drug was the first to be effective in the prevention of barbiturate poisoning after intravenous injection to dogs receiving simultaneous anesthetic and supra-anesthetic doses of pentobarbital sodium[12]. However, it is relatively unavailable and clinical data are lacking.

We have investigated the possibility of incorporating central stimulants into solid pharmaceutical preparations of barbiturates. If the corrective in question failed to show functional usefulness, its other properties were not investigated.

Nikethamide was eliminated because its most readily available form is unsuited for incorporation in solid pharmaceutical preparations of barbitur-

ates, also because its functional usefulness is doubtful (Table 1).

When caffeine was administered orally to dogs in doses of 150 mg./kg. simultaneously with 35 mg./kg. of pentobarbital sodium, convulsions developed without interfering with the depressant effect of this barbiturate. and a depressant. He showed that picrotoxin administered simultaneously with fatal doses of pentobarbital protected the animals. We have used this procedure to evaluate critically the protective action of several commonly used central analeptics as correctives. Table 1 shows that only picrotoxin and

TABLE 1.—TOXICITY IN MICE OF PENTOBARBITAL SODIUM ALONE AND WITH CORRECTIVE COMBINATIONS

Pentobarb. Na (mg./kg.)	*Corrective*			*Recovered*		*Died*			
	Drug	*Dose (mg./kg.)*	*No. mice*	*No.*	*Time† (min.)*	*No.*	*Time† (min.)*	*Mortality %*	*Corrective value*
150	None	0	43	1	573	42	12	98	
0	Amphetamine	40	6	6				0	
0	"	50	6	2	O.N.*	4	O.N.*	67	
0	"	150	5			5	O.N.*	100	
0	"	200	6			6	22	100	None
150	"	50	7			7	11	100	
150	"	150	7			7	5	100	
150	"	200	6			6	3	100	
0	Caffeine	600	3			3	11	100	None
150	"	600	6			6	7	100	
0	Dexedrine	50	3	3	O.N.*			0	
0	"	150	3			3	8	100	
150	"	50	6			6	9	100	None
150	"	150	3			3	3	100	
0	Nikethamide	450	3			3	7	100	
0	"	950	3			3	7	100	
0	"	1900	5			5	4	100	
150	"	450	4			4	25	100	None
150	"	950	6			6	13	100	
150	"	1900	2			2	2	100	
0	Pentylenetetrazol	200	10			10	4	100	
0	"	450	10			10	1	100	Marked
150	"	200	10	10	O.N.*			0	
150	"	450	46	36	300	10		22	
0	Picrotoxin	10	12			12	20	100	Definite
150	"	10	12	9	350	3	243	25	
0	Strychnine	5	6			6	7	100	
0	"	10	3			3	3	100	None
150	"	5	3			3	11	100	
150	"	10	6			6	19	100	

† Average time.
* Overnight.

When a combination of 125 mg./kg. of caffeine and 35 mg./kg. of pentobarbital sodium was administered, the dogs behaved exactly as the dogs receiving 35 mg./kg. of pentobarbital sodium alone.

Berger[2] employed a method of assay of central analeptics in mice involving the simultaneous intraperitoneal injection of a central stimulant pentylenetetrazol protect mice against one fatal dose of pentobarbital sodium. Caffeine, strychnine, nikethamide, amphetamine, and Dexedrine all failed to offer significant protection. In fact, in many instances the survival time appeared to be shortened. This is in agreement with results reported by Barlow[1] relative to the antidotal values of various analeptics in rabbits poison-

ed with sodium barbital. He found that nikethamide, strychnine, and caffeine were of questionable value in the treatment of even lighter grades of barbital poisoning. Occasionally the toxic effects of the antidote were additive with those of the hypnotic. Of all the analeptics studied, only picrotoxin and pentylenetetrazol offered sufficient protection to warrant consideration as barbiturate correctives. Because of its delayed onset of action in the central nervous system, picrotoxin may not be the corrective of choice for the majority of barbiturates of which the onset of action is instantaneous following absorption. It may prove to be more useful as a corrective for hypnotics that exhibit a similar delayed onset of action such as barbital or phenobarbital. Pentylenetetrazol appears to fulfill all the desiderata outlined for a suitable corrective of the central analeptic type (Corrective C). It is absorbed at approximately the same rate as the barbiturates, and its onset of action is approximately the same as that of the intermediate and short-acting barbiturates. In dogs its simultaneous administration with anesthetic doses of barbiturates (including phenobarbital) prevents or delays narcosis. Similar observations have been made in humans with reference to pentobarbital sodium[13].

Pentylenetetrazol is not irritating to the mucosa of the alimentary tract. It has been administered to humans in therapeutic doses (200 to 400 mg.) t.i.d. for several months without symptoms of gastrointestinal irritation[5]. In rats and in dogs, its daily administration for 90 days in doses of 90 mg./kg. produced no histopathological changes in the gastrointestinal tract, nor was there vomiting or diarrhea[16,22]. Vomiting may occur in dogs and in humans following large doses of pentylenetetrazol, but its delayed onset indicates a direct effect on the medullary vomiting centers rather than a local irritating effect on the gastrointestinal tract. This is further substantiated by the observation that intravenous injections of barbiturates promptly relieve such nausea, retching, and vomiting.

When pentylenetetrazol is combined with pentobarbital sodium or secobarbital sodium in the proportion of 3:1 and with phenobarbital sodium in a proportion of 3:2, it did not interfere with the effects of sedative or hypnotic doses of these barbiturates. This analeptic in small doses does not stimulate the sensorium: an effect that has been noted with caffeine and the sympathomimetic amines. In fact, it has been stated by Swenson and Grimes[24] that when pentylenetetrazol is administered to elderly subjects in doses of 60 mg. 3 times a day for a period of 30 days, their subjects experienced less difficulty in going to sleep and appeared to sleep in a more restful manner. Placebos administered in a similar manner produced no such effects. In our own experience, normal subjects given 0.5 to 1 gm. of pentylenetetrazol orally actually fell asleep while electroencephalographic tracings were being taken. Sleep spindles were noted in subjects who received from 500 to 700 mg. of pentylenetetrazol. As previously noted in dogs[13] and in mice (Table 1), there can be no doubt that pentylenetetrazol can lighten the depression and prolong the life of the experimental animals. It can save the lives of mice receiving an LD_{99} dose of pentobarbital sodium. In humans ingesting 45 mg./kg. of pentylenetetrazol and 15 mg./kg. of pentobarbital sodium (the critical ratio of 3:1), there was an appreciable delay in the onset of sleep. When sleep occurred, the subjects could be easily aroused. Thus, it is apparent that pentylenetetrazol does not nullify the effects of therapeutic doses (Table 2) but protects against overdoses of

the barbiturates in the above-recommended ratios.

In connection with the problem of duration of action, we have determined the average oral convulsant dose of pentylenetetrazol in dogs and in humans. There was not as much difference as might be expected. The average human oral convulsant dose of pentylenetetrazol is 45 mg./kg. while that for the dog is 75 mg./kg., although we have seen convulsions in a dog receiving 50 mg./kg. The onset of pentylenetetrazol action is rapid in both species, and its persistence of action depends on the size of the dose. The central excitatory effects of a convulsant dose of pentylenetetrazol may persist up to 8 hours in dogs. The duration of action of oral subconvulsive doses of pentylenetetrazol (20 to 40 mg./kg) was over 4 hours in humans, while that of convulsant doses extended over a period of 6 to 24 hours. Indications of the persistence of pentylenetetrazol effects are insomnia, excitement, retching, vomiting, and convulsions. Pentylenetetrazol, when taken orally in large doses, does not have the evanescent effect usually attributed to relatively small intravenous doses. A curious phenomenon with reference to the duration of action of pentylenetetrazol was encountered in mice receiving intraperitoneal injections of 450 mg./kg. of pentylenetetrazol combined with 150 mg./kg. of pentobarbital sodium. Following complete recovery from the barbiturate depression, an occasional mouse show-

TABLE 2.—COMPARISON OF SOPORIFIC ACTION OF PENTYLENETETRAZOL AND PENTOBARBITAL SODIUM WITH PENTOBARBITAL SODIUM ALONE

Patient No.	*Diagnosis*	*Sleep pattern*			
		*Tetropentobarb***			*Pentobarbital Na (100 mg.)*
		5/10/53	*5/11/53*	*5/12/53*	*5/13/53*
1	Myocardial infarction	Slept well	Slept well	Slept well*	Disturbed
2	Myocardial infarction	Slept well	Slept well	Slept well	Slept well
3	Myocardial infarction	Disturbed	Disturbed 2 capsules	Disturbed*	Disturbed*
4	Myocardial infarction	Slept well	Slept well	Slept well	Slept well
5	Myocardial infarction			Slept well	Slept well
6	Congestive heart failure			Slept well	Slept well
7	Pulmonary infarction	Disturbed			Slept well
8	Digitalis intoxication	Slept well	Slept well		
9	Carcinoma, lung	Slept well	Slept well	Slept well*	Slept well
10	Probable carcinoma, lung, with metastasis		Slept well	Slept well	Slept well
11	Tuberculosis		Slept well	Disturbed	Disturbed
12	Carcinoma, esophagus, terminal	Slept well 2 capsules	Slept well	Slept well	
13	Delirium tremens; alcoholism	Slept well	Disturbed	Slept well	Slept well
14	Alcoholism; emphysema		Disturbed	Disturbed*	
15	Fever, unknown origin	Slept well	Slept well	Slept well	Slept well
16	Carcinoma, stomach		Slept well	Slept well	Slept well
17	Possible meningitis		Slept well	Slept well	Slept well—Demerol q. 4 hr.
18	Arthritis		Slept well	Slept well	Slept well
19	Auricular fibrillation; fractured clavicle			Slept well	Disturbed

* Dosage repeated when patient failed to sleep.

** Tetropentobarb is a combination of 300 mg. of pentylenetetrazol and 100 mg. of pentobarbital sodium.

ed repeated tonic spasms indicating persistence of pentylenetetrazol action beyond that of pentobarbital sodium at this enormous dose level. In our experience the duration of action of orally administered pentylenetetrazol is such that it confers protection for a sufficient length of time to be of value as a corrective against barbiturate poisoning.

Sloane and Latven[22] administered pentylenetetrazol and its critical combinations with 3 barbiturates intragastrically to rats for 90 days. The dose of pentylenetetrazol alone was 90 mg./kg. for each rat. The dose of pentobarbital sodium was 30 mg./kg. in combination with 90 mg./kg. of pentylenetetrazol. The dose of secobarbital sodium was 30 mg./kg. in combination with 90 mg./kg. of pentylenetetrazol, and the dose of phenobarbital sodium was 45 mg./kg. in combination with 90 mg./kg. of pentylenetetrazol. No untoward or deleterious effects could be detected following gross examination or histologic study of the tissues of these rats. Livingston and James[16] performed similar experiments in dogs, administering pentylenetetrazol alone and in combination with secobarbital sodium for 90 days. They were unable to detect hematological or histopathological changes of the tissues (including the various portions of the central nervous system) following daily administration of 100 mg./kg. pentylenetetrazol alone and in combination with 33 mg./kg. secobarbital sodium.

The following combinations have been administered to human subjects daily for one year: 300 mg. pentylenetetrazol and 100 mg. pentobarbital sodium; 300 mg. pentylenetetrazol and 100 mg. secobarbital sodium; and 150 mg. pentylenetetrazol and 100 mg. phenobarbital sodium. No untoward effects have developed as judged by the behavior of the patients as well as by the periodic examination of their hemogram and urine[11].

As far as long-term human experiments indicate, there is no evidence that therapeutic doses of barbiturates in combination with pentylenetetrazol are either habit-formers or addiction liabilities. Certainly there is no evidence that even larger doses of pentylenetetrazol are addiction liabilities. There is no reason to expect that the combinations of pentylenetetrazol and barbiturates are more habit-forming or have greater addiction liabilities than barbiturates alone. The pioneer studies of Fraser *et al.*[7] should be repeated, using appropriate doses of pentylenetetrazol and barbiturates, to determine if this combination is an addiction liability.

It would appear that pentylenetetrazol meets the specifications for an ideal corrective more closely than any other available agent.

Discussion. Central stimulants (analeptics) have long been used in the treatment of acute barbiturate intoxication. Their employment, however, as additives (correctives) to pharmaceutical preparations of barbiturates in order to prevent or minimize the dangers of overdosage is the novel concept discussed in this paper. It has been common clinical experience that the prognosis of acute barbiturate poisoning to a great extent depends upon the time interval elapsing between the ingestion of the poison and the initiation of treatment: whether symptomatic or analeptic. As early as 1936, Gros and Hofmann[8] demonstrated that rats receiving an LD_{100} of barbital sodium recovered if given 400 mg./kg. of pentylenetetrazol 30 minutes following the administration of barbital. If the administration of pentylenetetrazol was delayed one, two, and three hours after barbiturate administration, the life-saving effect of pentylenetetrazol decreased as the

time interval increased. Their data are condensed in Table 3.

It should be obvious that the most appropriate time to administer central analeptics with barbiturates would be at the moment of their ingestion. Patients who take toxic or fatal doses of barbiturates may not be discovered for several hours, or even days, following the onset of barbiturate coma. A series of experiments was undertaken to determine whether the simultaneous administration of central stimulants and barbiturates would have a significant antidotal effect without interfering with the intended therapeutic effects of barbiturates. This was the case when pentylenetetrazol was employed as the barbiturate corrective. This antidotal effect might be inferred from the results of Gros and Hofmann[8]. We have shown that pentylenetetrazol protects against barbiturates whether given orally or intraperitoneally.

Orally-administered pentylenetetrazol in small doses does not have a strong central excitant effect; indeed, it tends to produce relaxation and sleep. That is probably the reason small amounts of pentylenetetrazol do not interfere with the effects of therapeutic doses of barbiturates. Perhaps pentylenetetrazol in small or moderate doses stimulates the sleep center; whereas barbiturates depress the center for wakefulness so that at low dosage levels no antagonism between these two drugs becomes manifest. Ogawa[19] noted that a weak central stimulant (para-hydroxycamphor) may by itself induce sleep even though it antagonizes barbiturate depression. In large doses pentylenetetrazol antagonizes the action of barbiturates wherever they act. This is in contrast to several other drugs which antagonize barbiturates only by selective stimulation of cortical and other specific centers.

There is little doubt that in order to counteract the effects of severely toxic doses of barbiturates, pentylenetetrazol must be administered in amounts capable of producing convulsions. This does not mean that convulsions should actually be manifest, but merely that the dose should be capable of producing convulsions in the absence of anticonvulsant concentrations of barbiturates in the central nervous system. The critical ratios between pentylenetetrazol and barbiturates must be based upon their mutual antagonism. When pentylenetetrazol was given to dogs in doses 4 times the convulsant dose combined with a single anesthetic dose of pentobarbital sodium only mild or no convulsions were noted.

The ratio of 3:1 proved to be satisfactory because after the administration of toxic doses of the combination, it prevented pentylenetetrazol convulsions as well as pentobarbital anesthesia. Apparently a similar protection holds for secobarbital sodium[16]. In the case of a combination of pentylenetetrazol and phenobarbital sodium, a

TABLE 3.—EFFECT ON SURVIVAL OF RATS OF VARYING THE TIME INTERVAL BETWEEN SUBCUTANEOUS ADMINISTRATION OF BARBITAL SODIUM (400 MG./KG.) AND PENTYLENETETRAZOL (400 MG./KG.)[8]

Time interval (minutes)	*No. rats used*	*No. rats surviving*	*Mortality %*
30	10	10	0
60	10	8	20
120	10	4	60
180	10	2	80

ratio of 3:1 was found to be too high. On a weight basis, phenobarbital is acutely less toxic than pentobarbital and also its onset of action on the central nervous system is slower. Our studies have indicated that the proper ratio is 3:2 for pentylenetetrazol and phenobarbital sodium. It may be inferred from the work of Gros and Hofmann[8] that the ratio of pentylenetetrazol to barbital sodium is 1:1.

In studies on human volunteers, the pentobarbital sodium-pentylenetetrazol combination was ingested by some subjects in doses of barbiturate up to 15 mg./kg. (total doses up to 18 grains). Some authors[14,23] have considered doses of this magnitude to be fatal. There are many patients hospitalized for acute barbiturate intoxication who consumed less than this dose. Assuming that 2 or 3 times the above dose of pentobarbital sodium represents the LD_{50} for humans, they would receive 2 or 3 times the average human convulsant dose of pentylenetetrazol if the recommended ratio of 3:1 is ingested. It is reasonable to assume that the protection seen with 45 mg./kg. of pentylenetetrazol vs. 15 mg./kg. of pentobarbital sodium would be manifest if these heroic doses of each drug were doubled or trebled.

This safety factor was demonstrated recently in a volunteer who took 12 Tetropentobarb capsules at one time (equivalent to 3,600 mg. of pentylenetetrazol and 1,200 mg. of pentobarbital sodium) during the daytime. Neither stimulation nor sleep was noted for 10 hours following ingestion, and normal sleep followed at night. No untoward effects could be detected at any time during close observation over a period of several days.

We do not know the mechanism of protective action of pentylenetetrazol or of picrotoxin. Since these drugs increase the cerebral metabolism which is depressed by barbiturates, it is likely that they compete with barbiturates for certain barbiturate-sensitive enzyme centers. This problem is being studied.

Summary and Conclusions. 1. Since the efficacy of administration of certain central analeptics varies as the time elapsing between the ingestion of barbiturates and the initiation of treatment, it is concluded that the simultaneous administration of such analeptics together with barbiturates would yield the best results. Hence, if a critical amount of analeptic is added to a given barbiturate preparation, a certain degree of protection must be conferred on a patient if he should ingest toxic or fatal doses of barbiturates.

2. Different types of correctives (additives to barbiturates) are described, and criteria established to warrant their clinical use.

3. Various analeptics commonly utilized in the treatment of acute barbiturate poisoning have been pharmacologically and clinically evaluated as to their efficacy as correctives.

4. At the present time only pentylenetetrazol and picrotoxin have been found to meet the criteria required for the ideal corrective.

5. It is presently believed that pentylenetetrazol is clinically and pharmaceutically the most suitable corrective for routine clinical use.

6. A critical ratio has been established for combining pentylenetetrazol with phenobarbital sodium at 3:2 and of pentylenetetrazol with pentobarbital or secobarbital sodium at 3:1.

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SURVIVAL RATES AFTER MYOCARDIAL INFARCTIONS WITH AND WITHOUT LONG TERM ANTICOAGULANT THERAPY

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Since coronary artery thrombosis is by far the commonest cause of myocardial infarction, prevention of this thrombosis should prevent most myocardial infarctions. Although many factors contribute to the likelihood of such thrombosis, the final event is the formation of the clot. The prevention of coagulation is an undertaking fraught with some hazard, and this is increased when anticoagulation is extended over a long period of time. The dangers are bleeding and its consequences, which are accentuated in the patient with coronary artery disease, in whom anoxemia due to anemia may be rapidly fatal[3]. Bishydroxycoumarin (Dicumarol) is the anticoagulant most often employed in long-term therapy, and until recently was most difficult to counteract, because a specific antidote was not available. It is now widely recognized, however, that vitamin K-1 emulsion used intravenously in small doses will quickly neutralize the hypoprothrombinemia due to bishydroxycoumarin (Dicumarol)[1,2,4]. This has made the long-range use of this anticoagulant immeasurably safer and more practical.

Following a reasonably cautious period of trial and observation, the medical profession generally has accepted the use of anticoagulants in the acute phase of myocardial infarction. The long-range use of these anticoagulants is still in this period of trial and observation. As a means of deriving some conclusions regarding such therapy, we have reviewed 2 comparable groups of patients with myocardial infarctions. The first group did not receive anticoagulants following the acute phase of their infarction; the second group did.

In both groups the diagnosis of myocardial infarction was confirmed by a 12-lead electrocardiogram. Most of the control group, who did not receive long-term anticoagulants, were cases diagnosed before the advent of such therapy. The patients who received long-term anticoagulants were selected for the following reasons: 1) Continuation of refractory anginal pain after recovery from infarction. 2) Survival of a recurrent myocardial infarction. 3) Development of coronary insufficiency suggesting impending coronary occlusion, following an asymptomatic post-infarction period. Only patients who fully understood their therapy and who could be relied upon to cooperate were chosen.

Bishydroxycoumarin (Dicumarol) is our choice for outpatient anticoagulant therapy. In addition to being less expensive than other oral anticoagulants,

it has been simpler to control because its effect is longer sustained, and because hazardous hypoprothrombinemia does not develop abruptly. Quick prothrombin times are performed every 7 to 14 days to determine the dosage of bishydroxycoumarin. Dosage varied from patient to patient, but we attempted to keep the prothrombin time about 25 seconds (40%). No patient was given outpatient Dicumarol who had not first had his general response to the drug determined during a 2-week hospitalization with close observation and daily prothrombin time determinations. No patient with a history of gastrointestinal or genitourinary bleeding, extensive liver disease, hemorrhagic diathesis, or other contraindication to anticoagulant therapy, was treated with outpatient anticoagulants.

Material. The control group of cases were chosen from the same time period as the treated cases, so that diagnostic criteria for infarction were the same for both groups. We believe variation in the extent of infarctions is similar in both groups.

For the purpose of analysis, both the control and treated groups were divided into those who had had a single myocardial infarction and those who had had recurrent infarction. In the control group there were 106 patients with single infarctions, and 41 with recurrent infarctions. In the group treated with long-term anticoagulants there were 31 cases with initial infarctions, 32 with recurrent infarctions.

Although many of these patients were followed for 3 or more years, the greatest percentage were followed either one or 2 years, and only these will be compared. All the 106 control patients with single infarcts were followed for at least 2 years. Of the 31 treated patients with single infarcts, all were followed for one year and 20 were followed for 2 years. Of the 41 control patients with recurrent infarcts, all were followed at least 2 years. Of the 32 treated cases with recurrent infarcts, all were followed one year, and 21 were followed a second year.

Only patients who survived the acute phase of myocardial infarction (6 weeks) are included in either the control or treated groups.

Results. In the control group with single infarcts, 13 of the 106 were dead at the end of the first year, and 13 more were dead at the end of the second year. By comparison, there was only one death among the 31 treated patients followed for one year, and no deaths among the 20 who were followed a second year.

In the control group with recurrent infarcts, 9 of the 41 were dead at the end of the first year, and 4 more died during the second year. By comparison, there were 2 deaths among the 32 cases of recurrent infarction who were treated with long-term anticoagulants for one year, and only one death among the 21 cases followed a second year.

Tables 1, 2, and 3 summarize these results by percentages. The smaller mortality in the treated group is impressive.

Chi^2 and significance levels for the tabulated results were determined by Dr. W. W. Mahan, our biostatistician, and are included in each table. The results in each group, except the follow-up of recurrent infarcts in the second year, were statistically significant for human data. In general it may be stated that approximately 4 times as many patients died without long-term anticoagulant therapy as did with it, over the period of observation stated.

Discussion. There are several factors in these series which add to their statistical significance. For example, the only patients with single infarcts who were placed on long-term anticoagulants were those who had continued pain, with the imminent probability of a second infarction. This made them a group with distinctly poorer prognosis than those who did not have such pain, and yet their survival rate was 3 times as good for 2 years, compared to that of the controls.

In addition, the one patient in the single infarct group treated with anticoagulants who did die had had some hemorrhagic complications which may have contributed to his death. While the risk of such complications is always present, it is much less than the over-all risk involved with the normal progress of the disease. This death occurred prior to the advent of vitamin K-1 vival data. In our experience over 30% of the patients with recurrent myocardial infarction die in either the immediate or acute phase. This is significant because it strengthens the case for protecting the patient who has had a single infarct.

Seven patients who were treated with long-term anticoagulants, but for various reasons had their therapy

TABLE 1.—SURVIVAL RATES OF MYOCARDIAL INFARCTS, FIRST YEAR OF FOLLOW-UP

		Single infarct	*Recurrent infarct*
Control	Survived	87.7% 106 cases	78.0% 41 cases
	Died	12.3%	22.0%
Treated	Survived	96.7% 31 cases	93.7% 32 cases
	Died	3.3%	6.3%
Statistical analysis		Chi^2 . . . 1.27 Level of significance, 30%	Chi^2 . . . 2.34 Level of significance, 15%

TABLE 2.—SURVIVAL RATES OF MYOCARDIAL INFARCTS, SECOND YEAR OF FOLLOW-UP

		Single infarct	*Recurrent infarct*
Control	Survived	86.0% 93 cases	87.5% 32 cases
	Died	14.0%	12.5%
Treated	Survived	100.0% 20 cases	95.2% 21 cases
	Died	0.0%	4.8%
Statistical analysis		Chi^2 . . . 1.93 Level of significance, 20%	Chi^2 . . . 0.22 Level of significance, 70%

TABLE 3.—SURVIVAL RATES OF MYOCARDIAL INFARCTS, TWO-YEAR FOLLOW-UP

		Single infarct	*Recurrent infarct*
Control	Survived	75.5% 106 cases	68.3% 41 cases
	Died	24.5%	31.7%
Treated	Survived	95.2% 21 cases	87.0% 23 cases
	Died	4.8%	13.0%
Statistical analysis		Chi^2 . . . 2.98 Level of significance, 10%	Chi^2 . . . 1.82 Level of significance, 20%

emulsion as a specific antidote for Dicumarol hypoprothrombinemia.

In the evaluation of the benefit derived from long-term anticoagulant therapy one set of statistics not included in this analysis is pertinent. That is the mortality in the immediate (3 days) and acute (6 weeks) phases of recurrent myocardial infarction. These deaths were eliminated from both the treated and untreated groups, since they are not part of the long-term sur- stopped, developed a new myocardial infarction soon after stopping their Dicumarol. Some of these patients were followed less than a year when their Dicumarol was discontinued and are not included in the survival statistics. Nevertheless, the development of new infarctions (which occurred from 2 to 12 weeks after cessation of anticoagulants) raises the question of whether the infarcts might have been prevented if the Dicumarol had been continued.

By contradistinction, 5 patients developed new myocardial infarcts while they were receiving long-term Dicumarol therapy. Four of these died and are included in the mortality statistics. The 4 who died all had some hemorrhagic complications during their therapy, necessitating blood replacement. All of them died of new coronary thromboses, and it is possible that the blood replacement therapy contributed to the pathogenesis of their thromboses, although this is speculative.

Our experience with the use of long-range Dicumarol now includes over 200 patients, manifesting various other thromboembolic complications, as well as those due to coronary artery disease. To calculate the incidence of hemorrhagic complications, one must first define such complications. If easy bruising, bleeding of the gums after vigorous brushing of the teeth, and so forth, are to be included, practically all our patients exhibited such phenomena at some time during their course of therapy. In patients who received anticoagulants for over 6 months, 10% experienced transient hematuria at some time or other. In addition to the 4 patients with coronary artery disease mentioned previously, 7 of the over 200 patients manifested what we consider to be major hemorrhage. Two of these had severe hematuria, one had major epistaxis, and 4 had melena and/or hematemesis. Of the latter, 2 were subsequently demonstrated to have a peptic ulcer. Although all 7 of these patients with major hemorrhage survived, more careful screening prior to the institution of therapy may have prevented their being placed on Dicumarol.

Summary and Conclusions. The use of anticoagulants on a long-term basis improved the prognosis of patients with either single or recurrent myocardial infarctions, over a 2-year period studied. Such therapy is associated with some hazard and should not be attempted unless the patient is carefully evaluated and the risks weighed against the possible benefits.

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STINGRAY INJURIES: A REVIEW AND DISCUSSION OF THEIR TREATMENT

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Although a most formidable venomous fish, the stingray is not usually considered to be belligerent. His sting, or caudal spine, while capable of penetrating the heavy cartilagenous jaw of a black-tipped shark[18], or transecting the thigh of a man[46], is probably seldom utilized except in self-defense either against those animals which feed upon him[5,18,27] or those humans who unfortunately tread upon him. In light of the studies made by Campbell[8], and from our experiences in handling hundreds of these fish both in and out of the water, it appears that they rarely, if ever, sting unless actively stimulated. There appears to be no evidence to support the assumption that these fish use their spines offensively either in obtaining food, or in attacking man. While Gudger[19] has felt that these fish do not sting bony fishes or other rays, it must be conceded that under unusual circumstances they may strike any animal which might be so unfortunate as to provoke their stinging responses[2,28].

Stingrays may reach a weight in excess of 700 pounds[20]. Fortunately, in those areas where they are prevalent, the species most likely to be encountered are of a relatively small size. Off the California coast the ray responsible for the greatest number of stingings is the rather small round stingray (*Urobatis halleri*), (Fig. 1). This animal reaches a length of about 50 cm.[38]; the sting of a 50 cm. ray will measure 6 cm. in length. These fish may possess a secondary sting which is also capable of inflicting an aggravating wound. The unbenevolent little round stingray lying half buried on the sandy bottom in the surf or the mud flats of the bay is often stepped upon by the unwary bather. The very weight of one's foot to the dorsal surface of this fish is sufficient stimulation for the animal to thrust his tail and sting upward and forward into the foot or leg of his victim. I have seen a fisherman suffer the same experience when placing a hopefully protected foot on one of these fish while attempting to dislodge his hook. A rubber boot is no measure of safety to be placed between one's anatomy and a stingray.

THE VENOM APPARATUS OF THE STINGRAY. It has been known since the days of antiquity that this fish possessed a caudal spine capable of inflicting an aggravating wound[1,36]. However, until a decade or so ago there was considerable question in the minds of some investigators regarding the presence of a venom-producing organ within the caudal structures of this fish. It is now generally agreed that along the ventral surface of the sting in the two "lateral grooves" lie certain specialized secretory cells which produce a toxin that is more directly responsible for the symptomatology experienced by the victim than is the mechanical injury caused by the sting.

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To Antonio Porta[37], 1905, and Muir Evans[13], 1916, we owe much for our present knowledge of the various anatomical components of this venom apparatus. The reader will find an excellent review of these papers in Gudger's[21] historical dissertation of the subject in 1943. A more recent investigation by Halstead and Modglin[25] of the sting of the California bat stingray is also recommendable. As seen in Fig. 2, the spine of a round stingray is

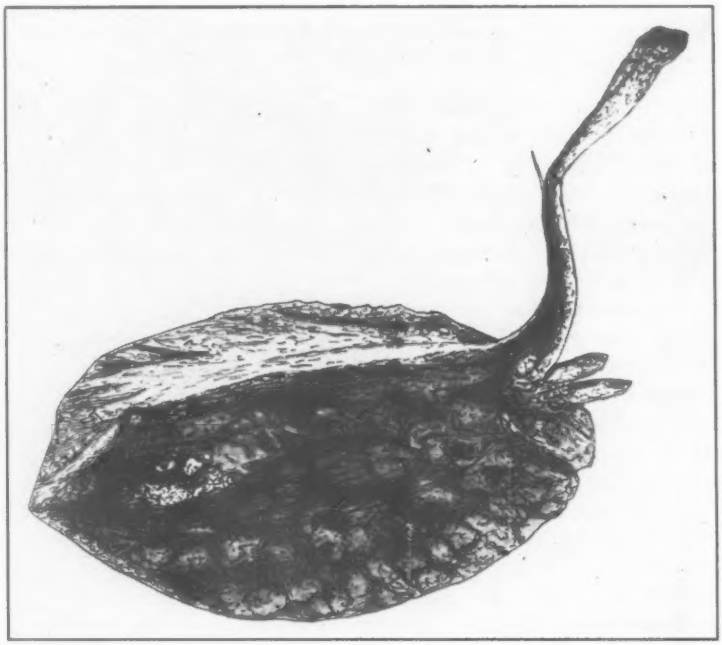

Fig. 1.—The round stingray.

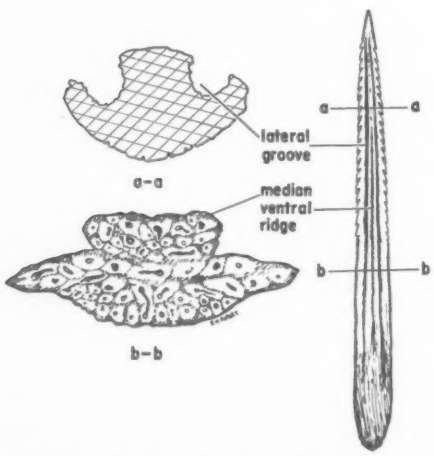

Fig. 2.—The spine of a stingray, showing the ventral surface and enlarged cross-sections.

a very treacherous looking weapon. The cross sections show the lateral grooves which embody the "glandular triangle" in which the venom is produced. Fig. 3 (Evans[14], 1924, Fig. 1a) depicts a glandular triangle in detail.

The sting itself is a calcified structure bilaterally serrated with sharp teeth along the distal half of its length. The teeth are curved cephalically and thus are responsible for the lacerating effect of the spine as it is withdrawn from the victim's flesh. When the spine is driven into the extremity of the victim, the pressure exerted by the involved tissues is sufficient to tear the integumentary sheath and to express the venom from the toxin-producing cells and their supporting structures. The secondary sting, when present, may or may not be calcified. If calcified, it usually resembles its larger and more dorsal counterpart both in its ability to produce a toxin and to inflict a wound.

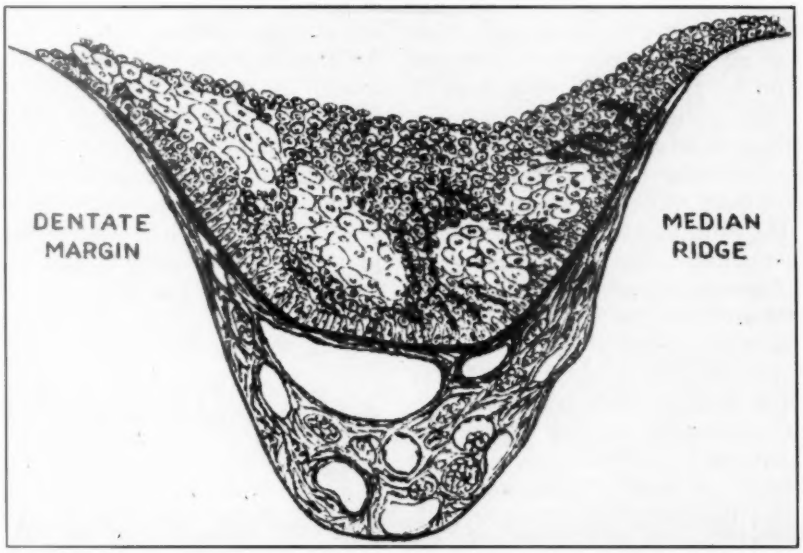

Fig. 3.—Enlarged cross-section of a lateral groove showing the glandular triangle. Superficially are the epithelial cells, some of which are distended with secretion. The deeper portion of the groove is composed of areolar connective tissue containing a number of blood vessels. The connective tissue is separated from the gland proper by a pigmented basement membrane. (Evans, courtesy of Phil. Trans. Roy. Soc. London.)

The Medical Problem. The increasing number of "stinger" victims referred to the physician through the lifeguard services along our beaches appears to confirm the assumption that these services view this problem with respect. During the 7-months' period between April and November of 1952, the lifeguard stations from Santa Monica, California, south to San Diego, California, a distance of about 130 miles, reported approximately 390 cases of stingray "attacks". Another 84 victims were treated by their physicians in areas unattended by the beach services. It is reasonable to assume that an additional 50 victims received no medical attention from either of the above sources and recovered from their wounds without particular incident. One-quarter of this total num-

ber of cases were seen by a physician at some time during the course of their recovery. The incident of secondary infection was exceedingly low, less than 3%. This was due in part to the early attention rendered by the lifeguard on duty or to his subsequent referral of the victim to a physician. It is the policy of the lifeguard services to advise medical attention for all persons stung by stingrays in which satisfactory recovery is not immediately apparent.

There appears to be considerable discrepancy in the none too abundant literature on the clinical syndrome of this entity. The principal reason for this lack of consistency is the failure on the part of some physicians to distinguish between those symptoms produced by the toxin and those derived from the mechanical effects of the sting itself. The number of deaths that may be attributed to the direct action of the venom is probably insignificant in comparison to the number of patients that have died in times past from the first-aid treatment or the secondary complications[12,29-32,41,43].

In 1931, Vellard[44] confirmed the toxicity of the venom by administering both crude extracts and fresh stings to animals. While Vellard's experiments are occasionally confusing, they demonstrate the toxicity of this venom *in vivo*. Since this original contribution several papers dealing with the effects of the venom have appeared. For the most part they deal with a review of the experimentally-induced symptomatology and offer no insight into the action of the substance itself. In 1952, at this institution, an investigation was made into the effects of the toxin on the various components of the cardiovascular system[40]. The results of this study indicate that in animals small amounts of the toxin may evoke a marked vasoconstriction following an initial period of vasodilatation. With increasing amounts, the substance affects the larger vessels, giving rise to changes in their tone. Finally, with still larger, and usually lethal doses, the venom may also act directly on the heart muscle, causing acute cardiac dilatation and failure. From these observations it should not be difficult to evaluate the symptomatology that has been recorded in the literature and to suggest a more rational approach to the treatment of the stingray victim.

Case Reports. The author has collected 29 case histories from victims stung by stingrays during the past 4 years. The clinical courses in most instances were similar, and the 4 cases herein presented represent an attempt to include the more significant complaints and findings. In the first two stingings, the author was present both at the time of, and immediately following the accidents.

CASE 1: A 27-year-old male employee of the California Department of Fish and Game was stung by a round stingray while seining in Alamitos Bay, California. He complained of discomfort almost immediately and within 2 minutes became aggravated by an intense, occasionally stabbing pain over the lateral aspect and ankle of the injured foot.

On examination, the patient was found to have received a freely bleeding wound of 2 cm. in length, approximately that distance below and slightly anterior to the lateral malleolus of the right foot. The wound could be probed to a depth of 1.5 cm. The leg was held in slight flexion; the ankle in flexion with some eversion. Both the flexor and extensor muscles of the foot were tensed. The patient went into primary shock but was quickly revived. At about 5 minutes following the onset of symptoms, sporadic muscular fibrillations could be seen along the length of the peroneus longus and brevis. The deep reflexes of the leg and foot were hypertonic; there were no pathological reflexes. Because of the intense local pain it was difficult to evaluate the sensory findings over the injured foot.

For some 30 minutes the patient complained of dizziness, nausea, and severe pain over

the right ankle and lower third of the leg. He continued to hold the injured foot in a flexed position even while reclining. Twenty minutes following the stinging, the pain had reached its greatest intensity. The blood pressure was 102/62 and the cardiac rate was 82. Following an unsuccessful attempt to control the pain with oral codeine, meperidine hydrochloride (Demerol), 75 mg., was given intramuscularly. The patient became relatively free of pain in about 15 minutes and was able to hobble about on the affected extremity. At this time the entire ankle was edematous and quite sensitive to pressure. The edges of the wound appeared ragged and somewhat purple in color. The laceration was then irrigated and a sterile nitrofurazone (Furacin) dressing applied.

Although some pain persisted for many hours, it was described as only occasionally sharp. It was further reduced when the extremity was soaked in hot water. By the following day most of the pain had subsided. The wound required about 16 days to heal, while the edema was apparent for the first 2 weeks of this period. The author is deeply indebted to this patient for allowing this observation of the symptomatology without the benefits of more intensive therapeutic measures.

CASE 2. A 37-year-old Mexican fisherman was stung on the right forearm by a 4-foot bat stingray (*Holorhinus californicus*), while attempting to dislodge the animal from his net. He received a 4-cm. laceration which could be probed to a depth of 3 cm. at its deepest point. The wound was immediately irrigated with salt water and within 3 minutes the patient was holding the extremity in hot water. The wound was kept immersed in hot water for 45 minutes. The patient complained of some localized pain and slight lethargy but exhibited no abnormal findings. On docking, the wound was cleansed, debrided, and closed with dermal sutures; sterile dressings were applied. During this entire period the victim was relatively free of pain and had no other complaints. The following day the dorsal aspect of the injured forearm was slightly swollen and tender but only to deep pressure about the wound. The patient was able to carry on routine dock work and experienced no other symptoms. The edema persisted for 3 days; the wound required 5 days for complete cicatrization.

CASE 3. A 9-year-old boy received two lacerations on the dorsum of the right foot in the area of the first metatarsal bone. The wounds were caused by a round stingray taken by a fisherman from Newport Harbor, California. The ray measured 40 cm. and possessed 2 stings, the larger of which was 4.4 cm. in length. The wound was "painted with iodine" and bandaged but because of the intense pain the victim was taken to a hospital one hour later. The wound was probed and a "small piece of slime" was removed from one of the lacerations. The youth's foot was swollen and extremely sensitive to pressure. The area about the wound was reddened and the skin temperature increased. The leg and foot were held in flexion and the patient grasped at his right knee while complaining of excruciating pain about his foot and leg. The patient was given an intramuscular injection of gr. 1/10 of morphine sulfate. Five minutes following the injection the patient became nauseated and thereafter vomited twice. The pain while somewhat alleviated was now described as being of a stabbing nature, confined for the most part to the area about the wound. Occasionally the patient would cry out from the "shooting pains" through his leg and thigh. There appeared to be little change in the intensity of the pain during the next 2 hours. The patient was given gr. 3 of calcium gluconate intravenously, penicillin 300,000 units and gr. 1 of phenobarbital.

Four hours following the accident, the patient complained of abdominal pain, nausea and pain on deep respiration; 10 minutes later he began to vomit. The author saw the patient at this time. The temperature was 99; pulse, 96; respirations, 24; blood pressure 85/60. The heart and lungs were normal to examination. The abdomen was flat, slightly rigid but without tenderness; peristalsis was increased. The right leg and ankle were held in flexion. There was no muscular atrophy or paralysis. Pitting edema extended over the entire dorsum of the right foot. The peripheral pulses were feeble. The deep reflexes of the right leg were hyperactive; the superficial reflexes were hypoactive. There were no pathological reflexes.

The patient was hospitalized and given 250 cc. of 5% glucose in water with 3 units of Eschatin added. The pain diminished during the following 12 hours and the recovery was uneventful. Twenty hours following the accident, most of the pain had subsided and the patient complained of only a dull ache about the site of the injury. Oral temperature was 99; pulse, 90; respirations, 18; blood pressure, 100/60. The edema persisted for 6 days, in spite of continual

elevation of the extremity. The wound was healed by the ninth day.

CASE 4. A 22-year-old female, employed as a dancer, was struck by the spine of a stingray while swimming in the surf off Seal Beach, California. The patient reportedly "fainted" several minutes later and was taken to an emergency hospital At the time of arrival, approximately 15 minutes following the stinging, the patient complained of severe pain in the injured foot, cramps in the ipsilateral leg, faintness, nausea and some dizziness.

Examination revealed a deeply lacerated, freely bleeding wound several centimeters in length, just anterior to the medial malleolus of the left foot. The edges of the wound were ragged and swollen. The patient held the foot flexed at the ankle and inverted. Temperature was 98.6°; pulse, 92; respirations, 20; blood pressure, 110/66. The lungs were clear and there were no cardiac abnormalities. The patellar and Achilles tendon reflexes on the affected side were hyperactive; there were no sensory changes.

The wound was cleansed and irrigated with a 5% solution of potassium permanganate. Some debridement was necessary. While the pain was confined to the area about the injury, the patient complained of severe cramps in the left leg and a numbness of the skin over the medial aspect of that foot. One hour following the accident, the pain was described as pulsating and intense. The patient was given gr. ¼ of morphine sulfate and gr. 2 of phenobarbital. Two hours later the patient was considerably improved and allowed to be taken home. Although the pain persisted for 24 hours, it was controlled with oral codeine. The area about the wound was deep red in color and edematous the following day. The localized edema and tenderness persisted for 11 days and the laceration was not well healed until the second week following the accident.

Comment. It is of interest to note that in 3 of these 4 cases the complaint of pain, out of proportion to that which might be produced by a similar nonvenomous injury, was immediate and unusually severe. This observation has been recorded by other investigators[3,4,15,17,25,41,46]. In 18 of the 29 cases in this study the pain appeared within 4 minutes following the accident. In 6 of the remaining 11 cases this complaint was registered within 10 minutes following the accident. The onset of pain was not usually limited to the point of injury but embraced an area of approximately 10 cm. in diameter about the wound. As the pain became more severe during the first 30 minutes, the area of complaint usually radiated to include a part of, or even the entire involved extremity. The pain appeared to reach its greatest intensity in less than 90 minutes and often persisted, though gradually diminishing in severity, for 6 to 48 hours. In 12 of the 29 patients there were complaints of "shooting pains" through the ipsilateral leg or arm. These seldom persisted for more than 8 hours.

From these observations it seems reasonable to assume that the intense nature of the pain is due primarily to the toxin produced by this fish. This is further substantiated by studies on experimental animals. When small amounts of the toxin are injected into the leg of a rabbit, the animal exhibits findings peculiar to the sensation of accentuated pain, namely vocalization, guarding, and increased blood pressure. While some of the victims in the present series suffered excruciating pain, the degree in most patients was not seemingly as severe as that which has been recorded in some other areas of the world. Fortunately the round stingray is quite small, and it may be that his venom is less virulent than that found in some of the other species of rays. However, I have been assured by many of the victims of the 29 cases in review that the pain they suffered was the most severe they had ever experienced.

Byam and Archibald[7], Colby[11], and others, have reported experiences with cases in which there was a definite paralysis affecting the injured extremity. The author has never seen either a spastic or flaccid paralysis in a stingray victim. While it may be possible for the venom to give rise to a true

paralysis, in the author's opinion the "spasms" so frequently reported, as in the present series of cases, are contractures initiated as flexion reflexes stimulated by the intense pain. Further evidence for this conclusion lies in the observation that all of the spasms were relieved by medications directed at alleviating the pain. An occasional paresthesia is sometimes noted about the affected extremity. This was not a constant finding in the present series and was questionably present in but 4 cases. It will be necessary to study a much larger group of patients before this complaint can be properly evaluated.

For the most part the symptoms of the venom are local manifestations. There is however, both clinical and experimental evidence to indicate that the toxin may produce systemic changes and even death. The nausea, faintness, vertigo and bradycardia so often experienced by the victim within the first few minutes after the accident may be attributed to the primary shock caused by the extreme pain. On the other hand, as pointed out by Russell and van Harreveld[40], there may be a fall in systemic arterial pressure due to peripheral vasodilatation when small amounts of the toxin are administered intravenously. It is conceivable that this and the related mechanisms may well accentuate, or even precipitate, a transient cerebral anoxia and shock.

Occasionally a patient may complain of pain on respiration (Case 2)[15], inguinal or axillary pain[41], or even generalized cramps[45]. Arrhythmias, tremors and increased nervousness, profuse sweating, diarrhea and vomiting with acute abdominal pain have all been reported as being associated with the effects of the venom. The frequency at which convulsions have been recorded however, both clinically and experimentally[26,41,44,45], indicates that this finding warrants more careful consideration. The mechanism responsible for the convulsive activity has not been identified in the literature. It would appear, from a study of the cardiovascular effects of the venom, that the convulsions may be the result of cerebral anoxia caused by the cardiovascular depression. Mice in which small doses of the toxin have been administered intraperitoneally exhibit findings typical of tissue anoxia: namely, cyanosis, blanching of the ears, changes in the respiratory excursions, ataxia, and others. If the degree of anoxia is increased by increasing the amount of the venom, the mouse may exhibit convulsive activity with either recovery or death. Whether the anoxia is due to a cardiovascular failure or to a histotoxic anoxia has not yet been determined.

The wound itself may be either of the laceration or puncture type. The penetration of the skin and the underlying tissues is usually made in a plane somewhat perpendicular to the skin's surface and is accomplished without serious damage to the surrounding tissues. In withdrawing the spine, however, extensive damage may be done by the serrated edges. I have seen a wound of 3.5 cm. in length perpetrated by a sting no wider than 5 mm. Cleland[10] tells of a fisherman who was stung by a 10-foot stingray off Australia. This animal struck with such force that he drove his sting through the lower third of the man's leg between the tibia and fibula leaving a wound 7 inches long on the lateral aspect of the leg and 4 inches long on the medial side. The wound edges are often jagged and not infrequently require debridement. The lacerated tissues bleed freely, though the bleeding does not appear to be of any greater intent than what one ought to expect from a similar non-venomous injury.

The degree of swelling about the wound varies, though it appears to be a constant finding. I have seen several patients in whom there was a marked pitting edema over the entire involved foot and lower third of the leg less than three hours following the stinging. The edema in some of these patients may often persist in spite of constant elevation of the extremity. Such edema is no doubt the result of the changes in the locally affected tissues. Whether or not the toxin is capable of evoking alterations in capillary permeability has not been demonstrated to the author's knowledge. There is reason to believe that much of the localized edema can be attributed to lymphatic obstruction, precipitated by inflammation, and damage to the lymphatic and supporting structures. These changes are due to the direct action of the venom.

The tissues about the injury often appear discolored leading to such descriptions as cyanotic[25], bluish[15], deep red (Case 4), or even purple (Case 1). While one would expect the lacerated edges of the wound to be discolored, this area often extends several centimeters from the injury. Further examination of these tissues reveals a definite necrosis which is probably due to the direct action of the toxin and its subsequent interference with the cells blood supply. In this respect the action of the toxin resembles the cytolytic and proteolytic effects of certain of the snake venoms.

Occasionally serious injuries may be inflicted by stingrays without the production of the symptomatology induced by the venom. Ronka and Roe[39] report a case in which a young man received multiple chest injuries from a ray which had driven one of its spines into the victim's right ventricular musculature and the other into local subcutaneous tissues. The authors relate no untoward effects from the venom. Following surgical removal of the spine, the patient recovered and was discharged from the hospital within 3 weeks of his injury. I myself was once stung by a stingray on the palmar surface of the distal third of the right index finger. I was quite surprised some time later to have experienced no acute pain or other local manifestations. On examining the sting responsible for the injury I discovered that the integumentary sheath with most of the glandular triangle had been lost previous to the stinging. As I sorted through the several hundred rays which we had taken in our seine I ascertained that over half of the stings were devoid of their sheaths and supporting tissues. These fish had lost most of the substance of the glandular triangle on the webbing of the net during the two hours in which they were being seined. It is quite possible that in some cases where the typical syndrome fails to develop this explanation may be applicable.

The description of the symptomatology and findings in this paper is by inference limited to the effects produced by the spine of the round stingray. While the discussion might be quite applicable to the wounds caused by other rays, it should be remembered that species differences may alter the clinical course of the entity. However there appears to be considerable similarity between the cases presented in this report and those observed by other writers dealing with different species of stingrays[3,4,7,11,12,15,17,21,22,25,35,41,45,46].

Treatment. The abundance of the number of remedies that have been suggested for the treatment of stingray injuries seems to indicate that the rationale of these therapeutic measures has not been overly obvious. While the larger portion of these suggestions have been recorded in the various zoological publications, some contributions have appeared in the columns

of the medical literature from time to time[3,6,15,23,29,31,32,34]. At the present writing there appears to be no therapeutic standard of procedure of which the physician might be advised. During the past year the author has been fortunate in receiving a large number of returned questionnaires, relating to the treatment of stingray victims, from physicians throughout the world. Many of the therapeutic proposals have received both laboratory and clinical trial, and while it is not within the scope of this thesis to discuss each measure, the more widely used agents and procedures will be considered in brief.

Any treatment to be successful must be instituted early and vigorously. The physician should direct his efforts towards:

1) alleviating the pain.
2) preventing complications that may be evoked by the venom.
3) preventing secondary infections.

Pain is by far the most frequent complaint for which the physician will be called upon to render medical aid. In this respect many local agents have been advised, including potassium permanganate, formalin, gold chloride, ammonium, adrenalin, antihistaminics, magnesium and calcium salts, alcohol, carbolic acid, fish liver, kerosene, tobacco juice and macerated cockroaches. In addition to these, other local measures are occasionally suggested: for example, incising and sucking the wound, cauterizing the lacerated edges, or packing the wound with leaves of certain plants.

With respect to these latter procedures several factors should be born in mind. The so called "incision and sucking" technique is of questionable value in some venomous wounds[9,42]. It would seem that such a technique in this particular type of wound would not only further mutilate the involved tissues but also create a greater surface area from which absorption of the toxin might be facilitated. This procedure also favors the development of secondary infections. Cauterization, while it may be effective, is both too drastic and usually too late to be practicable. The author once attended a seiner from the Gulf of California who having been stung by a sculpin applied a lighted cigarette to the wound. While the victim suffered no ill effects from the venom of this fish, he was later treated for a third degree burn and a secondary infection. The wound inflicted by this fish is of course of the pure puncture type and its treatment is not implied in this essay.

Potassium permanganate in solutions of .01 to 5% seems to have enjoyed considerable usage as an irrigant for relieving the pain of stingray injuries. It has been employed by both Gudger[24] and Evans[16], in whose hands it has reportedly been effective. Halstead[25] and Bayley[3] report indifferent successes with the solution, and in the experience of several physicians it has been found to be of little if any value (questionnaires present investigation). Potassium permanganate is a weak antiseptic whose chemical properties are well known. It was probably first suggested in stingray wounds because of its historical use in the treatment of certain snake bites. It is considered a useless remedy in the treatment of snake bites at the present time[33]. Until the chemical structure of the ray venom is known, the strength of Evans'[16] statement that the toxin may be readily destroyed by permanganate of potash, rests on questionable clinical data and no experimental evidence. I do not use potassium permanganate myself, because I have not found it effective clinically, nor have I yet been able to demonstrate either *in vitro* or *in vivo* that the aqueous solution destroys the venom. It is not unlikely that the credit often given to the

permanganate should be bestowed upon the mechanical effects of the irrigating process or the hot water with which it is often administered.

Formaldehyde in solutions of .05 to 40% have been used for irrigating the lacerated tissues. A fisherman from the Gulf of Mexico once showed me a 4-cm. scar on the dorsum of his right foot. The scar had been caused by a strong solution of formaldehyde the fisherman had applied to a stingray injury of 1 cm. in length. Formaldehyde is a good disinfectant, but its use in the treatment of venomous injuries should be avoided. "Ammonia water" at present appears to have commandeered a considerable number of advocates. It is used, in hot water, by several physicians and lifeguard services. Dr. Arthur Christensen of Glendale once attended a patient who had received both an epispastic and caustic effect from a strong solution of ammonia water which had been applied by a "first aider" to a stingray injury. The patient was unable to decide whether the venom or the treatment was the more aggravating. Ammonia, too, can be forfeited for a more simple and rational procedure.

The use of locally injected anesthetics has been advocated by several writers[3,47]. This procedure may have a very definite place in the treatment of venomous injuries. However, it is often limited by the fact that the region of complaint in many victims may extend over an area too great for the efficacy of the injection. Procaine hydrochloride "blocks" are occasionally used by physicians in Australia and New Zealand for this type of injury. The author once obtained a good result with an intravenous infusion of 5 cc. of 1% procaine in 15 cc. of isotonic sodium chloride. The solution was administered over a period of 3 minutes and seemed to afford relief to the patient for approximately 50 minutes.

The author has found the following treatment to be the most successful. Until some further insight into the chemistry of the toxin is gleaned, these measures appear to be adequate. As the degree of success depends largely upon the rapidity at which the therapy is instituted, the ideal treatment would be one instituted by the patient himself. The wound should be irrigated immediately with the cold salt water at hand, not only may much of the toxin be washed out by the mechanics of this operation, but the cold water serves both as a vasoconstrictor and mild anesthetic agent. In the hands of a physician, or one properly qualified, a constriction band may be applied immediately above the stab site. When the wound has been thoroughly irrigated and no evidence of the sting's epithelial sheath can be seen, the extremity may be submerged in hot water. The water should be maintained at as high a temperature as the patient can tolerate without injury, for 30 minues to an hour. The addition of various anesthetic and antiseptic agents to the hot water is optional. The author has suggested to the lifeguard services that they might add magnesium sulfate to the water, because of its mild anesthetic properties. Following the soaking procedure the wound should be further debrided, cleansed and closed with dermal sutures if necessary. An antiseptic and sterile dressing should be applied to the injured area. Preliminary studies have indicated that heat may have a detrimental effect on the venom; boiling readily destroys the toxin *in vitro*. While hot water alleviates the pain produced by the venom, it is not clear whether the mechanism responsible for this effect is one involving changes in the chemistry of the toxin, or one exclusive of such changes.

If this treament has been instituted early and effectively, there may be no need for administering antibiotics as evidenced in the low rate of secondary infections in the present series. Some physicians, however, recommend penicillin and the appropriate anti-tetanus agents routinely following stingray injuries. Occasionally, when all local measures have failed to relieve the pain, or when absorption of the toxin has progressed beyond the applicability of such treatment, the use of intramuscular or intravenous Demerol has been found to be very effective. In those cases where irrigation is contraindicated (where the spine has entered the abdominal or thoracic cavity), the patient should be hospitalized and observed closely following the necessary surgical procedures. The primary shock so often seen immediately following the stinging usually responds to simple supportive measures. However, the secondary shock which occurs as a result of, or from, the direct action of the venom on the cardiovascular system, warrants immediate and strenuous therapy. Treatment in any such case should be directed toward maintaining cardiovascular tone and the prevention of any further complicating factors.

Summary. The stingray is a venomous fish capable of inflicting an aggravating injury by means of its sting or caudal spine. The symptomatology experienced by victims stung by these fish may be attributed to the venom contained within the ventrolateral grooves of the animal's stinging apparatus and/or to the mechanical injury perpetrated by the sting.

While the chief complaint of the patient is usually pain, it must be remembered that the venom may evoke cardiovascular changes. Clinically the circulatory effects are usually limited to peripheral changes. It is conceivable however, that where large amounts of the toxin may be absorbed, complete cardiovascular collapse may ensue.

The importance of early and vigorous treatment is emphasized. Such treatment should be instituted towards alleviating the pain, preventing complications that may be evoked by the venom and preventing secondary infections.

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1-HYDRAZINOPHTHALAZINE (APRESOLINE) IN THE TREATMENT OF HYPERTENSIVE DISEASE: A CLINICAL TRIAL WITH A CONTROL GROUP*

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This is a study of the effect of 1-hydrazinophthalazine in the treatment of ambulatory patients with hypertensive disease. The result of this treatment, alone, is compared to the treatment of a similar group of patients with a placebo. The alternate patient method was used in determining which patient was to receive the drug or the placebo.

Hydrazinophthalazine has been reported by other observers to produce a hypotensive effect in animals and in man[1,2,4-6,9-11]. The purpose of this study was to discover whether there would be a difference between the drug and placebo groups that was statistically significant over a period of several months.

The mechanism of action is believed to be central in origin by several investigators[2,3,12,13]. This would seem to be borne out by a number of clinical and laboratory facts. When administered, the drug does not produce nasal congestion nor does it reverse the action of epinephrine, as might be expected if the site of action was at the peripheral nerve ending. Also, it does not appear to block ganglionic transmission as there are no pupillary changes. Likewise, there is no potentiation of epinephrine and/or norepinephrine. The bradycardia of norepinephrine is blocked which suggests interference of the central reflexes mediated through the carotid sinus and aortic arch as a response to hypertension. Taylor, Page and Corcoran[13] postulate that the action is that of inhibition of a cerebral humoral factor as has been demonstrated in dogs by cross circulation experiments. In this study, the circulation of the central nervous system cephalad to C5 of a dog (Dog A) was isolated from the circulation of the rest of the body and perfused by another dog. The effluent jugular blood of the head of Dog A was returned by an anastomosis to the jugular vein of Dog B. Both vagi of Dog A were cut in the neck but the cord and sympathetic chains were intact. The central end of one or both vagi of Dog A was stimulated with a sinusoidal current of an intensity of 50 milliamperes. Stimulation carried on for 2 to 6 minutes resulted in a slow rise of arterial pressure in Dog B which slowly disappeared after stimulation was stopped. Apresoline blocked this pressor effect.

Schroeder believes the action is to antagonize the action of several humoral factors, such as pherentasin, serotonin and norepinephrine[3,11-13].

This study confined itself to the use of 1-hydrazinophthalazine alone in one group of patients, compared to the use

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of identically appearing placebos in another group. The time that these patients have been followed is inadequate to determine any ultimate change in the progress of their disease. It has been sufficient in most, however, to determine whether the drug has or has not affected their blood pressure for a number of months. The use of a control group was deemed necessary in view of the difficulty in evaluating any new drug in the treatment of hypertension, a condition which is many times altered by the patient's subjective response to medical attention.

Method. The subjects were followed on an out-patient basis. Patients were referred for study from the medical clinic of Los Angeles County General Hospital. The only criteria for the selection of patients, both control and those to receive Apresoline, were a diastolic blood pressure of 95 mm. Hg or over, a normal nonprotein nitrogen and no history of angina pectoris or coronary occlusion. Old age or a history of cerebral vascular accident did not exclude patients from the study.

Prior to the onset of treatment, all patients had an electrocardiogram taken, a urine analysis, a complete blood count, and a nonprotein nitrogen determination. These determinations were repeated at intervals during the study.

The patient's history was noted, and a complete physical examination, including funduscopy, was done at the initial visit. In alternate fashion these patients were started on either Apresoline or placebo.

All patients given the drug were started on a dose of 100 mg. daily (25 mg. q.i.d.) by mouth. Dosage was increased by 100 mg. per day at weekly intervals until the desired effect was obtained. The maximum amount of medication given to any individual patient was 800 mg. in 24 hours.

At each subsequent visit, the blood pressure was taken in the supine and sitting positions, the patient having rested in that position for at least 10 minutes prior to the reading.

These patients were seen at intervals ranging from one week to one month.

Several patients were not included in this series but did receive Apresoline inasmuch as their precarious situation demanded some intervention. Likewise, a few other patients who were hospitalized during treatment are not included. These cases will be discussed briefly later.

Material. Thirty-nine clinic patients were started on Apresoline. Two patients in this group failed to return after the initial visit and hence are not included. The remaining 37 patients were maintained on the drug from periods ranging from one to 15 months.

Thirty-seven patients were placed on placebos which were identical in appearance to Apresoline. Nine were eliminated because of failure to maintain attendance at the clinic leaving a total of 28 patients who continued on the placebos for periods varying from one to 14 months. In one case, the pressure in the supine position, only, was measured. Thus, there were 27 patients with both supine and sitting pressures.

All patients were seen by the same observer at each clinic visit and all blood pressures were taken by this same individual using the same sphygmomanometer.

In order to preserve consistency, the diastolic pressure was taken at the point at which the sounds disappeared.

Results. From the accompanying tables it will be observed that both groups of patients revealed some lowering of blood pressures. The percentage decrease in systolic pressure in the great majority of patients in both groups exceeded the percentage fall in diastolic pressure.

A glance at Tables 1, and 2 and Fig. 2, points out that those patients receiving Apresoline show a more significant hypotensive effect, both systolic and diastolic, than their control counterparts.

The average systolic fall in the Apresoline group was 26.3 mm. Hg as compared with a fall of 12.4 mm. in the control group. Similarly, the average diastolic fall of 14.3 mm. Hg in the Apresoline group compared to a 4.4 mm. drop in the control patients.

Those patients in each group who evidenced a diastolic pressure less than 94 mm. Hg at the time of their last visit are indicated (see Tables 1 and 2). There were 5 such patients in the control section and 9 in the Apresoline-treated group (17.8% and 24.3

TABLE 1. PATIENTS ON APRESOLINE

Case	Age	Diagnosis	Time followed in months	Decrease in systolic pressure mm. of Hg		Percentage decrease in systolic pressure		Decrease in diastolic pressure mm. of Hg		Percentage decrease in diastolic pressure		Final diastolic below 95 mm. Hg	Discontinued due to side effects	Hypertension known	Symptomatic change			
				Supine	Sitting	Supine %	Sitting %	Supine	Sitting	Supine %	Sitting %				Worse	Same	Sl. imp.	Much imp.
1	38	Ess. Hyp. I	5	20	40	9.5	17.4	14	18	10.0	12.0			10 yrs.		x		
2	60	Ess Hyp. I	6	60	60	27.1	25.0	20	20	18.2	18.2	x		2 yrs.		x		
3	48	Ess. Hyp. I	2	60	22	16.6	13.0	24	18	20.4	15.2			3 yrs.			x	
4	53	Ess. Hyp. I	2	28	20	25.0	11.1	28	22	23.6	18.6		x	1 yr.		x		
5	60	Chr. Pyeloneph.	2	50	20	24.5	0	28	+20	23.6	+18.2			6 mos.			x	
6	41	Chr. Pyeloneph.	2	10	40	16.6	21.0	16	15	13.3	14.0			14 yrs.		x		
7	61	Ess. Hyp.	2	62	10	25.6	4.7	12	14	10.8	14.0		x	7 yrs.	x			
8	37	Chr. Pyeloneph.	2	20	50	12.5	29.4	28	26	25.5	25.5	x	x	11 yrs.	x			
9	61	Chr. Pyeloneph.	4	38	40	12.4	18.2	24	24	22.2	22.2	x		1 yrs.			x	
10	39	Chr. Glomerulo.	1	48	46	20.0	19.2	10	6	7.7	4.6			2 yrs.			x	
11	50	Ess. Hyp.	2	30	38	13.7	16.8	+2	20	+1.7	15.4		x	10 yrs.		x		
12	39	Ess. Hyp.	1	0	20	0	8.7	12	0	9.2	0			7 yrs.	x			
13	56	Ess. Hyp.	4	34	30	16.0	16.0	20	34	15.4	25.5		x	5 yrs.		x		
14	58	Ess. Hyp.	2	30	20	13.0	9.5	10	14	10.0	13.5	x		2 yrs.				
15	46	Ess. Hyp.	2	40	50	20.0	23.6	20	25	16.6	20.0		x	3 yrs.		x		
16	42	Ess. Hyp.	4	10	12	5.6	7.1	0	0	0	0			3 yrs.				x
17	25	Chr. Pyeloneph.	4	20	20	6.6	13.4	18	18	16.4	16.6	x		3 yrs.	x			
18	42	Ess. Hyp.	3	10	0	4.5	0	+4	6	+3.1	4.6		x	2 yrs.	x			
19	43	Ess. Hyp.	1	28	6	13.5	3.9	6	2	4.7	1.6			1 yr.		x		
20	61	Ess. Hyp.	1	0	14	0	9.2	2	12	2.1	13.0	x	x	6 yrs.	x			
21	37	Ess. Hyp.	1	20	20	10.9	10.5	44	16	27.5	11.0			7 mos.				
22	35	Ess. Hyp.	6	20	30	12.5	18.9	28	32	23.5	26.2	x		7 yrs.		x		
23	42	Ess. Hyp.	4	30	60	12.0	20.5	10	30	7.7	20.0		x	3 yrs.	x			
24	32	Ess. Hyp.	2	52	20	26.0	10.0	20	4	16.6	5.0			4 yrs.	x			
25	31	Ess. Hyp.	3	4	22	2.4	12.8	4	10	3.2	7.8			4 yrs.	x			
26	49	Ess. Hyp.	1	70	30	27.0	13.0	20	10	14.2	4.5			10 yrs.		x		
27	27	Ess. Hyp.	3	+10	14	+5.9	+8.2	+5	+10	+4.5	+10.0		x	11 mos.				
28	41	Chr. Glomerulo.	3	10	30	5.5	14.2	60	42	46.0	32.0	x		0		x		
29	60	Ess. Hyp.	6	20	40	11.1	10.5	18	21	18.0	20.0	x		8 yrs.		x		
30	63	Ess. Hyp.	6	70	40	25.0	15.4	40	26	25.0	17.2			15 yrs.			x	
31	66	Ess. Hyp.	5	25	50	13.5	25.0	20	0	16.6	0			1 yr.		x		
32	36	Ess. Hyp.	6	20	30	9.1	13.1	0	22	0	14.5			9 yrs.			x	
33	47	Ess. Hyp.	2	+20	0	+11.1	0	+10	14	+9.2	11.8			12 yrs.				
34	65	Ess. Hyp.	2	30	30	12.5	12.5	16	10	14.5	9.1			10 yrs.				
35	42	Ess. Hyp.	2	30	40	13.5	17.5	0	10	0	7.6		x	1 yr.	x			
36	57	Ess. Hyp.	1	38	12	19.0	6.6	10	2	9.6	2.0			7 yrs.	x			
37	31	Ess. Hyp.	3	12	+16	6.3	+10.0	12	+10	8.6	+8.4			14 yrs.	x			
		Averages		26.3	26.4	12.2	12.1	14.9	13.7	12.0	10.8							

+ before a figure means a rise

TABLE 2.—PATIENTS ON PLACEBOS

Case	Age	Diagnosis	Time followed in months	Decrease in systolic pressure mm. of Hg		Percentage decrease in systolic pressure		Decrease in diastolic pressure mm. of Hg		Percentage decrease in diastolic pressure		Final diastolic below 95 mm. Hg	Hypertension known	Symptomatic change				Case
				Supine	Sitting	Supine %	Sitting %	Supine	Sitting	Supine %	Sitting %			Worse	Same	Sl. imp.	Much imp.	
1	50	Ess. Hyp.	3	12	16	6.0	8.4	+2	+10	+1.5	+8.3		12 yrs.			x		1
2	68	Ess. Hyp.	3	10	10	4.7	5.0	0	10	0	8.3		20 yrs.		x			2
3	50	Ess. Hyp.	3	10	10	4.0	3.8	10	20	8.3	15.3		35 yrs.		x			3
4	52	Ess. Hyp.	4	30	16	13.6	7.4	16	12	13.3	10.6		20 yrs.			x		4
5	61	Chr. Pyeloneph.	2	6	0	3.5	0	6	4	6.0	4.0	x	6 yrs.	x				5
6	61	Ess. Hyp.	9	10	20	5.9	11.7	10	8	10.0	8.0	x	2 mos.		x			6
7	74	Ess. Hyp.	6	0	30	0	13.6	0	+2	0	+1.7		10 yrs.			x		7
8	70	Chr. Pyeloneph.	2	0	20	0	10.0	2	10	2.2	10.0	x	8 yrs.		x			8
9	48	Ess. Hyp.	2	+10	10	+5.2	5.2	0	4	0	3.2		3 yrs.			x		9
10	40	Ess. Hyp.	3	10	40	4.5	18.1	8	0	5.8	0		25 yrs.			x		10
11	39	Ess. Hyp.	2	10		5.0		2		1.6			1 mo.				x	11
12	69	Ess. Hyp.	4	8	10	4.4	5.0	20	10	16.1	6.7		4 yrs.		x			12
13	66	Ess. Hyp.	5	+10	+2	+5.5	+1.1	5	2	4.7	1.8		5 yrs.		x			13
14	55	Ess. Hyp.	4	16	28	10.0	16.4	6	6	6.0	6.0	x	1 yr.		x			14
15	32	Ess. Hyp.	6	14	0	8.5	0	8	+12	8.1	+12.2		1 yr.		x			15
16	50	Ess. Hyp.	2	30	30	14.3	14.3	+30	4	+27.2	3.2		2 yrs.		x			16
17	32	Ess. Hyp.	5	+26	0	+14.4	0	0	10	0	7.1		3 yrs.			x		17
18	74	Ess. Hyp.	2	+20	+10	+11.1	+4.7	16	6	13.3	5.4		35 yrs.			x		18
19	65	Ess. Hyp.	6	14	40	+7.6	17.4	15	5	14.2	5.7		2 yrs.		x			19
20	59	Ess. Hyp.	3	0	14	0	7.8	0	2	0	1.5		15 yrs.			x		20
21	64	Ess. Hyp.	7	10	20	5.5	10.5	6	4	5.4	3.6		3 yrs		x			21
22	56	Ess. Hyp.	4	20	10	8.6	4.3	10	0	8.3	0		2 yrs.			x		22
23	50	Ess. Hyp.	2	30	40	14.3	18.2	10	18	8.3	15.4		7 yrs.	x				23
24	75	Ess. Hyp.	3	34	26	15.5	11.7	14	20	14.0	18.5	x	6 yrs.	x				24
25	68	Chr. Pyeloneph.	7	30	80	14.4	33.2	16	26	13.3	21.5		7 yrs.			x		25
26	57	Ess. Hyp.	4	50	20	19.2	8.4	+5	+20	+4.5	+18.2		20 yrs.				x	26
27	67	Ess. Hyp.	6	+20	0	+9.1	0	0	0	0	0		2 yrs.		x			27
28	55	Chr. Pyeloneph.	6	+8	+30	+5.0	+17.5	+10	+23	+9.8	+21.8		2 yrs		x			28
		Averages		9.3	16.2	4.2	7.6	4.7	4.0	4.1	3.4							

+ before a figure means a rise

respectively).

An additional study was made of the patients in each group who had been on either drug or placebo for 6 months or more. Figure 2 presents these patients with each line representing the percentage change in supine, diastolic pressure in a patient.

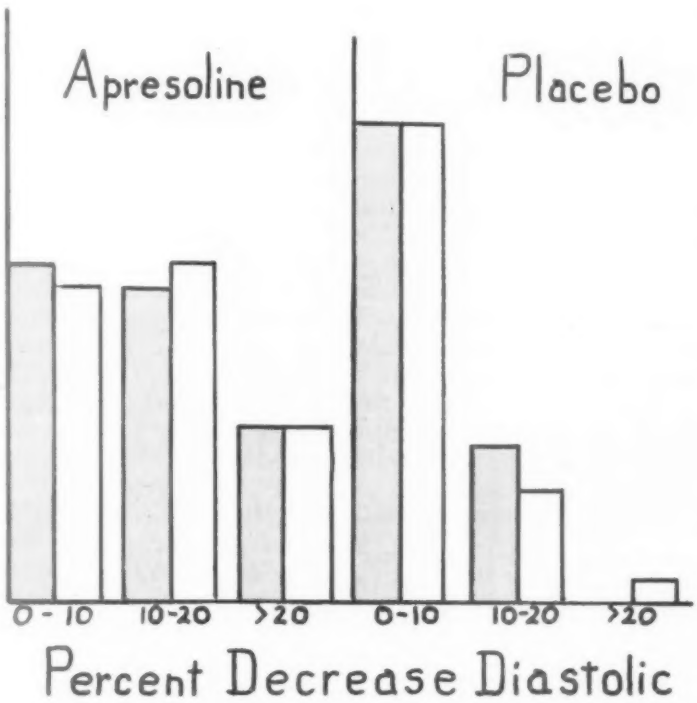

Fig. 1.—The patients in Tables 1 and 2 are divided according to the % decrease in their diastolic pressures during the time of the study.
The shaded bars represent their supine diastolic pressures while the clear bars represent their sitting diastolic pressures.
The 0-10 bars include all those patients who had a rise in pressure in the interval of time studied.

There were 18 such patients in the placebo group and 15 such patients in the Apresoline group.

The actual fall in blood pressure and the percentage decreases given represent the differences between initial pressures and the final blood pressure reading taken. Transient decreases with a return to previous levels are, therefore, not revealed by these charts. Four patients in each group had an increase in their diastolic blood pressure.

STATISTICAL ANALYSIS. Since the diastolic pressure is generally considered to be of greater significance in hypertension than the systolic, only the percentage drop in diastolic pressure has been treated statistically here.

The purpose of a statistical analysis of the data in this study is to determine whether or not the drug, Apresoline, lowers the diastolic pressure significantly over that which would be expected if the drug had not been given at all.

Because the difference between the drop in diastolic pressure supine and the drop in diastolic pressure sitting is not a constant factor, each was treated separately.

In general, the curves for the placebo group (both sitting and supine) show a tendency to be skewed in the direction of minimal drop. Such a tendency can be reasonably expected, because it stands to reason that in the group in which no effective medication is being given, the pressures should tend to show no change.

On the other hand, both the supine and sitting pressures of the Apresoline group show less marked tendencies to skew, and in fact if the extreme value on each end of the drug supine group is discarded, that distribution would be nearly normal. Such results would seem to indicate what ordinarily would be expected: that Apresoline effects a drop in pressure proportionate to the individual's susceptibility to it, and that the drop in pressures of a sufficiently large group of people would tend to fall along a curve of normal distribution.

The results are as follows:

cannot be due to chance alone. The fact that significant changes are still measurable after the drug has been in use for 6 or more months indicates that the drug still exerts an effect even after long continued use.

Although a smaller number of people stayed on for over 6 months, the placebos show the same tendency to skew as does the larger group, while the drug group is more nearly normal in distribution.

There is no significant difference between the drop in supine and the drop in sitting pressures in the Apresoline group. The same is true for the placebos.

In determining whether or not the placebo group differs significantly from 0, a t value of 1.80 is obtained for the sitting distribution and 2.48 for the supine. Neither of these values is significant, again a probability of 0.01 being considered the lower limit of significance.

There are not enough males in the original group to make an adequate sex difference study. Also there are no significant correlations between the placebo and the drug groups for age

	PLACEBOS		APRESOLINE		
Position	*No. of cases*	*Placebos M and S°*	*No. of cases*	*Apresoline M and S°*	*t value*
SITTING	27	$3.5 \pm 9.9°$	37	$11.1 \pm 10.5°$	2.93
SUPINE	28	$4.1 \pm 8.8°$	37	$12.1 \pm 10.6°$	3.29
SUPINE–Over 6 Months	18	$3.6 \pm 6.4°$	15	$14.1 \pm 12.6°$	2.84

$$°\text{standard deviation} = \sqrt{\frac{\Sigma(x-\bar{x})^2 - [\Sigma(x-\bar{x})]^2/n}{n-1}}$$

x = individual measurement
$\bar{x}$ = working mean
n = No. of cases

The t values in each case are significant, a probability of 0.01 being considered the lower limit of significance. Thus, it can be concluded that the differences between the patients treated with placebos and the Apresoline

of the patient or duration of hypertension prior to therapy. Much larger sample sizes are needed for such correlations.

SIDE EFFECTS. As has been previously reported, headache, usually occipital,

was the major unpleasant side effect. Nearly every patient on Apresoline developed this symptom. The headache did abate, even though the medication was continued in most instances, but it sometimes took several weeks to do so. Tachycardia was frequently encountered but was usually much shorter in duration than the headache. Vomiting was seen 10 times, and 6 of these patients had other severe reactions such as headache, fever, or marked tachycardia. Eleven of the 37 patients on Apresoline had to discontinue the drug because of the severity of these side effects.

Two patients who had received Apresoline for many months showed a slow progressive decline in their total white blood count to levels of 2,400 and 2,600 respectively. On continued treatment, on the same dose of Apresoline, their counts gradually returned to pre-treatment levels which were low initially in both instances (5000 and 4800). The differential counts remained normal as did their hemoglobin values.

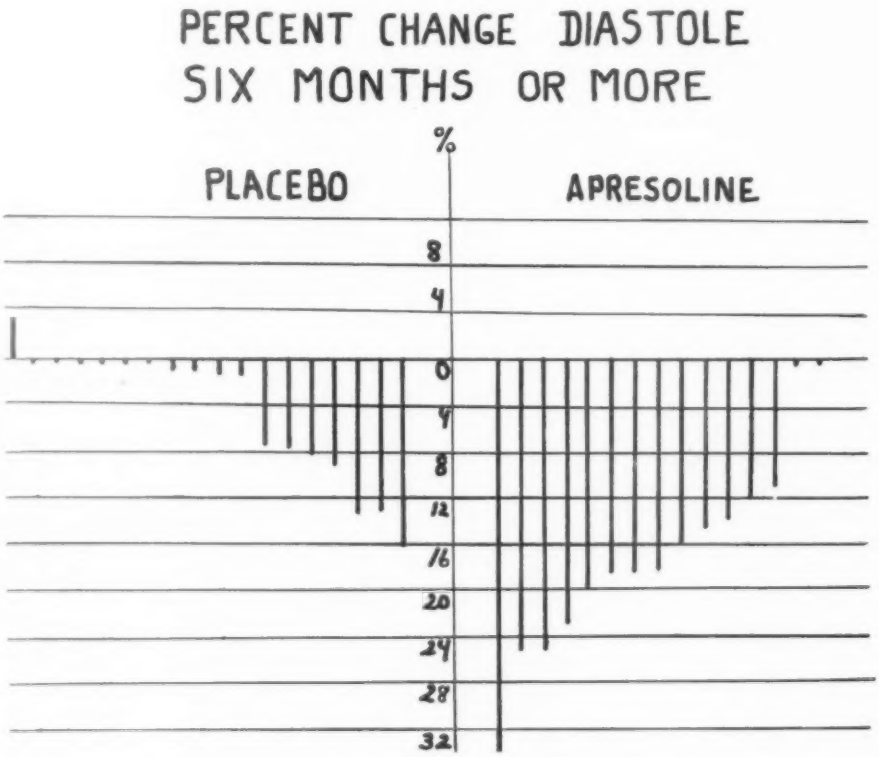

Fig. 2.—Each line represents the % change in supine diastolic blood pressure of a patient followed for more than 6 months.

Two patients on placebo, who did not return after one visit, were found to have discontinued them due to headache and nausea, which they felt were drug induced.

SPECIAL PATIENTS. Of the patients who were not included in the regular clinic series, 4 had undergone previous

sympathectomy with return of persistent hypertension. Of these only one responded to Apresoline and his diastolic pressure was reduced from 124 to 100 mm. Hg.

Two patients had malignant hypertension with papilledema and diastolic pressures of 180 mm. and 120 mm. Hg respectively. The former had a drop in diastolic pressure to 138 and was then subjected to surgery. The latter had a drop in diastolic to 90 mm. Hg and clearing of eye ground signs of pathology, but refused to continue the drug due to nausea and vomiting.

The last patient of this group was a patient diagnosed as toxemia of pregnancy, who had previously lost a child during an eclamptic convulsion. This patient had a return of her blood pressure to normal levels and delivered a normal, full-term child uneventfully.

Of the patients treated in the hospital, 3 had chronic renal disease and nitrogen retention. The downhill course in all 3 appeared to be hastened by the administration of Apresoline.

Discussion. The difficulty in obtaining 2 comparable groups of patients with hypertensive disease is evident by the greater severity in the patients receiving Apresoline. It is not feasible to grade the patients according to Smithwick's classification because the patients were not in the hospital and did not have a sedation test or a phenolsulfonphthalein determination. None of the patients in the series had nitrogen retention, none of them had eye ground changes worse than a grade III by the Keith-Wagener-Barker classification, and none were in congestive failure.

Schroeder treated 50 patients with doses ranging from 100 to 800 mg. per day, for from one to 40 weeks. He obtained sustained decreases of blood pressure in 35. In 20 with neurogenic hypertension, he states the drop was 33 mm. Hg systolic and 25 mm. Hg diastolic. In 8 patients with renal hypertension, the average decrease was 25 mm. Hg systolic and 16 mm. Hg diastolic. In 9 with malignant hypertension, the decreases were 37 mm. Hg and 30 mm. Hg[10].

Page, in treating 70 patients with Apresoline on both a hospital and outpatient basis, found 5 were unable to take the drug, 33 had a fall of 20 mm. Hg or more, 14 had an equivocal fall, and 17 had none.

From our study, it may be seen that the response to Apresoline varies greatly from one patient to the next, so that average figures in such a series mean very little.

Some patients had a good response to Apresoline alone, some had none. Both groups of patients apparently improved by regular visits to the same physician, showing the beneficial effect on blood pressure of a doctor-patient relationship.

The side effects of Apresoline, principally the headache, are a definite difficulty in its administration. The headache is felt by Schroeder[11] to be histamine-like and the use of antihistaminics has been recommended for it. Some of these patients responded to antihistamine but more did not, and they had to weather the storm of the first few days or weeks before the headaches subsided.

The 3 hospitalized patients with nitrogen retention, appeared to be adversely affected by Apresoline, so that its use in uremia may be contraindicated. The drug was tried on these 3 patients in the hope that an increased renal blood flow would benefit them. Even though renal hyperemia has been demonstrated with Apresoline, the filtration fraction usually decreases and the glomerular filtration rate changes insignificantly[5-8].

In view of the reports on the increased susceptibility of patients who had sympathectomies to Apresoline[3,9,11],

the results here were surprising in that only one patient out of 4 had a response.

In the future many of these patients are to be tried on one of the ganglionic blocking agents, and then a combination of the 2 drugs.

Summary and Conclusions. A group of 37 patients with hypertensive disease were treated with Apresoline on an out-patient basis. Of these, 12 had a drop in diastolic pressure of 20% or more, either while supine or sitting. Eleven patients discontinued the drug due to side effects. The principal severe side effect was an occipital headache.

Another group of 28 patients with hypertensive disease received identically-appearing placebos. Of these, one patient had a drop in diastolic pressure, while sitting, of more than 20%. Two patients discontinued taking placebos because of headache and nausea. Those patients followed more than 6 months, demonstrate that the significant difference in placebo and Apresoline persists after many months.

Uremia appears to contraindicate the use of Apresoline. Placebo medication will sometimes lower blood pressure. Apresoline is an agent worth trying in the treatment of hypertensive disease.

At this time it does not seem to be possible to predict which patient will respond.

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MODIFICATION OF THE HYPOTENSIVE ACTION OF 1-HYDRAZINOPHTHALAZINE (APRESOLINE) BY PRISCOLINE, HEXAMETHONIUM AND SECTION OF THE CAROTID SINUS AND AORTIC BUFFER NERVES IN NORMAL AND RENAL HYPERTENSIVE DOGS

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A search for agents that enhance the hypotensive action of Apresoline would be rewarding if they increased the effectiveness of Apresoline in the treatment of essential hypertension. This study measures the modifying action of 2 drugs, 2-benzylimidazoline HCl (Priscoline) and hexamethonium, on the acute hypotensive response to intravenous Apresoline in normal and renal hypertensive dogs; the effect of states of altered activity of the carotid sinus and aortic buffer reflexes is also determined.

Methods. Arterial pressure was recorded by a mercury manometer on a smoked drum from a cannulated femoral artery of adult mongrel dogs anesthetized with sodium pentobarbital (30 mg./kg.). Renal hypertension was elicited by the perinephritis method of Page[1], and neurogenic hypertension by section of the carotid sinus and aortic buffer nerves. Drugs were injected through a needle inserted into a femoral vein. Apresoline (1 mg./kg.) was given by quick injection to normal and renal hypertensive dogs; retesting was done at intervals of not less than one week and the modifying action of either Priscoline or hexamethonium on response to Apresoline determined on separate occasions. The effect of section of the carotid sinus nerves one or more weeks prior to testing was then measured, following which the modifying effect of hexamethonium and Priscoline was remeasured as before. Retesting was done under as nearly identical conditions as possible.

Results. These are summarized in Table 1.

HYPOTENSIVE ACTION OF APRESOLINE IN NORMAL, NEUROGENIC AND RENAL HYPERTENSIVE DOGS. Apresoline (1 mg./kg.) usually produced only slight fall in arterial pressure in normal anesthetized dogs, and there was no correlation between magnitude of the response and the control arterial pressure level. Arterial pressure began to decline 1 to 2 minutes after the injection; responses were maximal usually within 8 to 12 minutes and arterial pressure remained at or near this level an average of 20 to 30 minutes.

Hypotensive responses to Apresoline were essentially the same in renal hypertensive as in normal dogs, though somewhat larger responses were present in 2 experiments. But neurogenic hypertensive dogs responded with large and prolonged falls in arterial pressure, even when control arterial pressure levels were the same, or but slightly higher than in anesthetized normal dogs.

INFLUENCE OF PRISCOLINE ON THE HYPOTENSIVE RESPONSE TO APRESOLINE. Priscoline was given intravenously in doses of 4 mg./kg. until pressor responses to injection of 10 gamma of noradrenaline were largely prevented or mainly reversed (total dose of 12

to 16 mg./kg.). Apresoline was not given until the initial sympathomimetic action of Priscoline was mainly over and decline in arterial pressure seemed maximal. Priscoline enhanced the hypotensive response to Apresoline in 4 of 5 normal dogs and in 2 dogs with renal hypertension, despite somewhat lower resting arterial pressure level after its administration.

EFFECT OF SECTION OF THE CAROTID SINUS NERVES ON HYPOTENSIVE ACTION

TABLE 1.—HYPOTENSIVE ACTION OF APRESOLINE IN ANESTHETIZED NORMAL, NEUROGENIC AND RENAL HYPERTENSIVE DOGS AND MODIFYING EFFECT OF PRISCOLINE, HEXAMETHONIUM AND SECTION OF THE CAROTID SINUS NERVES

Normal dogs	*Hexamethonium (2 to ¼ mg./kg.)*	*Priscoline (12 to 16 mg./kg.)*	*Section carotid sinus nerves*	*Hexamethonium (2 to ¼ mg./kg.)*	*Priscoline (12 to 16 mg./kg.)*
1. 184		170	182	135	148
−22		−88	−81	−18	−88
2. 158		136			
−13		−38			
3. 120	106		135	77	
−24	−22		−51	−25	
4. 129		98	126	104	115
−13		−43	−42	−32	−49
5. 182		166	181	150	153
−88		−76	−66	−36	−89
6. 166		122			
−7		−31			
7. 150			152	124	
−12			−66	−12	
8. 182			147		
−7			−69		
9. 187	178				
−40	−25				
10. 182	142				
−32	−27				
11. 164	114				
−36	−4				
Renal Hypertensive					
1. 226	190	186	228	146	
−64	−21	−80	−106	−46	
2. 196	142		218	154	122
−72	−14		−94	−43	−54
3. 250					
−12					
4. 190					
0					
5. 180					
−6					
6. 222	227	165			
−22	−18	−67			
Neurogenic Hypertensive					
1. 190					
−116					
2. 186					
−87					
3. 160	113				
−82	−20				
4. 178					
−98					
5. 240					
−146					
6. 250					
−126					

Top number represents control arterial pressure; number beneath represents hypotensive response to Apresoline. All values in mm. Hg.

OF APRESOLINE. Section of the carotid sinuses between ties to ensure division of the carotid sinus nerves one or more weeks prior to testing caused marked augmentation of the hypotensive response to Apresoline in both normal and renal hypertensive dogs. Combined administration of Apresoline and Priscoline, as before section of the carotid sinus nerves, resulted in lower arterial pressure levels than administration of either drug alone.

INFLUENCE OF HEXAMETHONIUM ON HYPOTENSIVE ACTION OF APRESOLINE. Intravenous hexamethonium (2 to 4 mg./kg.) decreased, or did not change, the depressor response to Apresoline in both normal and renal hypertensive dogs. Arterial pressures were lower after hexamethonium but they were usually no lower, or not as low, as after Priscoline, and the latter drug augmented rather than diminished the depressor action of Apresoline.

When hypotensive responses to Apresoline were increased in normal and renal hypertensive dogs by section of the carotid sinus nerves, administration of hexamethonium decreased the enhanced response, contrasting again with the effect of Priscoline.

Administration of Apresoline (1 to 3 mg./kg.) did not enhance the hypotensive responses to single doses of 5 mg. of hexamethonium in normal dogs, but the combined effect of the 2 drugs resulted in slightly lower arterial pressures than administration of either alone.

Comment. The acute hypotensive action of Apresoline in dogs was not augmented by hexamethonium, and Apresoline did not enhance the hypotensive action of hexamethonium; their effects were additive; no synergistic action between the 2 drugs was found. Priscoline, on the other hand, lowered arterial pressure and also augmented the hypotensive action of Apresoline, with which it presumably acts synergistically. Thus, these results suggest that combined therapy with Priscoline and Apresoline would be more effective in the treatment of hypertension than use of either drug alone, or use of hexamethonium and Apresoline together.

Renal hypertensive dogs were not more sensitive to Apresoline than normal dogs, which implies that Apresoline does not specifically oppose a mechanism mediating renal hypertension, and this is in accord with clinical experience[2]. Neurogenic hypertensive dogs were much more responsive to Apresoline and this is also in accord with clinical experience[2] since Apresoline more effectively lowers arterial pressure in patients with hypertension believed to be predominantly neurogenic.

A point of interest lies in the observation that section of the carotid sinus nerves alone, which did not cause hypertension or tachycardia, greatly increased the hypotensive response to Apresoline. Such change in responsiveness might provide an approach to the treatment of the too frequent severe hypertension unresponsive to usual therapeutic regimens, including Apresoline.

Summary. The acute hypotensive response to intravenous Apresoline is unchanged or diminished by prior treatment with hexamethonium and enhanced by Priscoline in normal and renal hypertensive dogs; response to hexamethonium is unchanged or diminished by prior treatment with Apresoline. The combined effect of Apresoline and hexamethonium is additive while the combined effect of Apresoline and Priscoline is greater and probably represents a synergistic action. Sensitivity to Apresoline is not increased in renal hypertensive dogs compared with normal dogs; sensitivity is increased in neurogenic hypertensive dogs. Section

of the carotid sinus nerves alone did not result in hypertension or tachycardia but did markedly enhance the hypotensive action of Apresoline.

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EVALUATION OF TREATMENT IN HYPERTENSION: EFFECTS OF CINCHONA ALKALOIDS*

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The cinchona alkaloids have long been known to produce circulatory depression when administered parenterally in large amounts. This has indeed been viewed as an undesirable property of the drugs. However, recent studies have shown that these alkaloids may lower the arterial blood pressure when given by mouth in well-tolerated amounts. This was noted by Hiatt's group in dogs with "neurogenic" hypertension[7], though not in normal dogs[9] nor in those rendered hypertensive by renal artery clamps[6]. Similarly, Ferrer *et al.*[5], during an investigation into the effects on the circulation of single oral doses of quinidine, observed a transient but marked fall in blood pressure in man. In fact, the lowering of the blood pressure, which occurred in both normal and hypertensive persons, was the most consistent result of quinidine therapy. A reasonable basis for this effect can be developed from the vasodilatation[5,7,9] and adrenergic blockade[8] known to follow administration of cinchona compounds.

In light of this evidence, a study was instituted to determine the efficacy of quinidine and quinine as long-term therapy in human hypertension. It was felt that the usual procedures for evaluating hypotensive drugs do not adequately control personal bias, the effects of treatment *per se*, and the spontaneous variability of the blood pressure. Therefore, this study was based upon the "double-blind" principle, by which the effects of a drug are compared with those of a placebo

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and neither the patient nor the attending doctor is aware of the nature of therapy.

Method of Study. The study was restricted to patients with long-standing hypertension. At the first visit they were divided at random into 2 groups, to receive 4-week courses of quinidine sulfate and placebo in alternate order. Observations were made at weekly intervals for changes in symptoms and blood pressure. At the end of this period, the patients were followed without therapy for at least 2 weeks, then redivided at random into 2 groups and given 4-week courses of quinine sulfate and placebo under the same conditions.

PATIENT MATERIAL. The test population consisted of 44 patients drawn from the cardiac clinics of the Hospital for Joint Diseases and the Beth Israel Hospital. All had been under treatment for at least one year, most with a diagnosis simply of essential hypertension. An average of 8.1 pressure determinations were available for each patient from the previous year in the cardiac clinics; the mean pressure for the group being 193/109. No attempt was made to classify the cardiovascular abnormalities but all patients satisfied the following criteria; the mean blood pressure for the previous year and at the first study session was above 150/90, normal sinus rhythm was present, and there was no evidence of valvular lesions nor congestive failure. Thirteen patients (34% of the group) had histories of prior failure but were maintained in compensation during this project. The cardiac regimens had previously been stabilized and were not altered during the study. Eight patients were maintained on mercurials while 5 required digitalis and mercurials. Self-administration of other drugs was discouraged and the patients were questioned routinely for this possibility. It developed that 10 patients took occasional doses of other medication; nitroglycerine in 3, Priscoline in one, and barbiturates in 8.

The ages of the patients ranged from 43 to 77 with an average of 58 years. Thirty-five were female and 9 male. Only one was free of complaints throughout the study. The common symptoms, each of which occurred in at least 20% of the cases, were, in order of decreasing frequency: headache, palpitation, weakness, chest pain, nervousness, dizziness, tinnitus, and vague visual disorders.

TEST PROCEDURE: Patients were usually seen by the same doctor at each visit. The subjects remained seated throughout each study session. After several minutes rest, the patients were asked to evaluate the preceding week as "better," "worse," or "the same," by comparison with a "usual" week. They were then questioned for evidence of cinchonism and the severity and frequency of individual symptoms were determined. The physician in turn evaluated the week as a whole before the blood pressure was taken. This was measured with a Tycos aneroid sphygmomanometer following the procedure outlined by the American Heart Association[3]. Pressure readings were made 6 times at each visit, using both arms alternately with a minimum of 10 minutes time between readings on each arm. The importance of this maneuver to accurate measurements was demonstrated early. The mean for all patients of the first reading at the initial session was 201/115; the mean of the sixth reading was 187/112. One woman developed a fall in pressure of 75/24 in the approximately 25-minute interval. The average for all readings at the first visit was 191/113, a figure which corresponded closely to the mean of all readings for the previous year. At the end of each visit, medication for the next week was given.

MEDICATION. Quinidine sulfate, quinine sulfate, and the lactose placebo were dispensed in identical capsules, identified only by code number. Each capsule contained 0.3 gm. of a drug. Physicians in contact with patients were not informed of the contents of the capsules. The patients were told that the test materials were "medicine for the blood pressure" and were instructed to take 3 capsules a day, one before each meal. This dose (0.9 gm. per day) was considered to be the maximum amount of alkaloids which could be taken regularly by an unselected group of patients, an opinion substantiated by later experience. They were questioned at each visit as to the number of capsules actually ingested. Sixty-six per cent of the subjects took the full number of 21 capsules per week during both the quinidine-placebo and quinine-placebo courses. For the group as a whole, the doses actually taken each week averaged 19.8 during the quinidine study and 20.0 in the later quinine phase.

HANDLING OF DATA. Since only one week's supply of drugs was dispensed at a time, each acceptable test period required at least 2 consecutive weekly visits. When appointments were broken or patients took grossly inadequate numbers of capsules, the next weekly visit was omitted from the study and the particular drug administered for an additional week. Such occurrences were not usual

during the first phase. Sixteen of the 44 patients went through treatment with quinidine and placebo in consecutive weeks and the group required a mean of only 9.0 weeks to complete the 8 treatment weeks. Cooperation was poorer in the quinine-placebo phase. Only 2 of the 18 patients completed this course without interruption and the average time needed to obtain 8 acceptable weeks was 16.8 weeks.

Data were summarized by one who had no contact with patients. The blood pressure for the first week on each drug was excluded from calculations, to accentuate any effects which might require time to appear. Thus the average treatment blood pressure for each individual was based upon the 18 readings taken during the last 3 weeks of administration of each test material. The original blood pressure was taken as the mean of the 6 readings at the first visit, before administration of any treatment. Average values and standard deviations for the group as a whole were derived from the means of individuals. Differences between means both for individuals and groups, were analyzed by the Fisher "t" test and differences were held significant if the P value was .05 or less.

Results. EFFECTS OF QUINIDINE. All 44 patients received alternate 4-week courses of quinidine sulfate and placebo. Twenty-one received quinidine followed by placebo. In the other 23 the order of administration was reversed.

Blood Pressure. The blood pressure under quinidine therapy fell from the original mean for the group of 191/113 to an average of 174/103. This mean fall of 17/10 mm. Hg was statistically significant for the group as a whole. When patients were considered individually, the effect was more striking. A statistically significant lowering occurred in the systolic pressure in 24 patients (55%) and in the diastolic in 26 (59%). In these patients manifesting significant reductions, the average fall was 30/16 mm. Hg and the maximum lowering was 79 points systolic and 48 diastolic. In only 3 instances did the systolic pressure while on quinidine rise above the original level and the diastolic pressure was never raised.

However, the placebo largely duplicated the effects of quinidine. The mean blood pressure during administration of lactose was 177/105, also representing a statistically significant fall from the initial level. On an individual basis, the lowering of the pressure was significant for 24 patients (55%) in the systolic range and 27 (61%) in the diastolic. The average reduction in these selected patients was 24/12 and the maximum 59/35 mm. Hg.

Therefore, it became necessary to consider not only the effects of quinidine on the blood pressure, but the effects of quinidine contrasted to those of placebo. The experiment was designed to permit such analysis, since each patient while receiving the placebo acted as his own control for treatment with the alkaloid and the original blood pressures could be ignored. Two facts became obvious from this comparison of the 2 agents; the blood pressure declined after either therapy was started and whichever drug was given second was more effective (Table 1). The average pressure of 174/103 for the group while on quinidine was not essentially different from that of 177/105 during placebo administration. However, when individual patients were considered, the majority showed statistically significant differences between the pressures on quinidine and on placebo (Table 2). In 13 the systolic readings were lower on placebo and in 10 lower on quinidine. Unless a specific hypotensive action is attributed to lactose, this difference must be due only to chance. The diastolic pressure was lower in 18 on quinidine and in 8 on placebo. This greater lowering of the diastolic readings, particularly in those patients given quinidine as the second drug (Tables 1 and 2), was the only possibly significant difference between the 2 agents. The importance of this is difficult to evaluate. If

chance governed the distribution it would be expected that equal numbers of patients would have been bettered by quinidine and placebo, or that equal numbers would fall into the categories of "quinidine superior," "placebo

efficacy of quinidine was probably not meaningful.

It is of incidental interest that the blood pressure 2 weeks following cessation of therapy was lower than that observed under treatment. The failure

TABLE 1.—EFFECTS OF QUINIDINE AND PLACEBO ON MEAN BLOOD PRESSURE

		By order of administration	
	Total	*Quinidine first*	*Quinidine second*
Number of Patients	44	21	23
Systolic Pressure			
Original[1]	191 ± 24	187 ± 21	194 ± 26
Quinidine[2]	174 ± 22	173 ± 19	175 ± 25
Placebo[2]	177 ± 22	171 ± 20	182 ± 23
Final[3]	171 ± 24	169 ± 24	172 ± 24
Diastolic Pressure			
Original[1]	113 ± 15	115 ± 16	111 ± 14
Quinidine[2]	103 ± 15	106 ± 16	99 ± 13
Placebo[2]	105 ± 14	105 ± 16	105 ± 12
Final[3]	102 ± 16	103 ± 18	101 ± 15

Values expressed as mean ± standard deviation for groups of patients
[1] from 6 readings on each patient at initial visit
[2] from 18 readings on each patient during last 3 weeks while receiving drugs
[3] from 6 readings on each patient 2 weeks after drugs stopped

TABLE 2.—COMPARISON OF EFFECTS OF QUINIDINE AND PLACEBO ON BLOOD PRESSURE OF INDIVIDUAL PATIENTS

		By order of administration	
	Total	*Quinidine first*	*Quinidine second*
Number of Patients	44	21	23
Systolic Pressure			
Quinidine "superior"	10	4	6
Placebo "superior"	13	9	4
No difference	21	8	13
Diastolic Pressure			
Quinidine "superior"	18	6	12
Placebo "superior"	8	7	1
No difference	18	8	10

Mean pressure while on quinidine therapy compared to mean pressure on placebo therapy in each patient. Differences between means evaluated by Fisher "t" test and considered significant if P value .05 or less. Numbers tabulated are the numbers of patients in each category.

superior" and "no difference." These hypotheses were compared to the observed distribution by the quantity chi square method. By this procedure, the results were not found to be significantly different from a chance distribution. Thus the slightly greater

of the pressure to rise may be taken as another indication that the hypotensive actions were not induced by the drugs administered but stemmed rather from the treatment situation.

Symptomatology. The patients reported moderate improvement in well-

being from quinidine administration. Forty-one percent of the patient-weeks were considered to show definite improvement and only 23% were rated as worse than usual. Again though, quinidine must be compared with the placebo. On lactose, the patients classified 38% of the weeks as better and 23% as worse. (Table 3). It was of interest that the physician's evaluation agreed with the patients' opinion less than 2/3 of the time. In general the doctor was more conservative, feeling that improvement occurred in 28% of the quinidine weeks and 26% of the placebo weeks.

It was also of interest, in regard to the reliance which may be placed upon subjective symptoms in a study of this kind, to note the dissociation between the patients' opinions of weeks as a whole and their opinions of changes in specific symptoms. It was a common occurrence to have a patient rate a particular week as considerably improved and then report an increase in the severity and frequency of every symptom. The reverse also occurred less frequently. As shown in Table 3, there was no real difference in effects on symptoms between the 2 materials tested. If the total weekly reports on all symptoms were considered, there was improvement in 30% of symptoms on quinidine and 33% on placebo. Generally, headache and palpitations were most improved, weakness and dizziness least. The frequency with which placebos relieved headache is of particular interest in view of the number of anti-hypertensive measures recommended for control of this symptom.

Finally, there was a total lack of correlation between changes in the blood pressure and changes in symptoms. For example, 9 patients may be considered as showing the most improvement in the blood pressure with statistically significant falls from the original levels in both the systolic and diastolic pressure while on both quinidine and placebo. The pressure reached normal levels in 3 of these. Nevertheless, only 29% of the total symptoms were improved and the patients themselves considered only 40% of the weeks to have been benefited. These results were no different from those obtained in patients with little or no change in the blood pressure.

Toxicity. A marked incidence of side-reactions developed. Many patients originally intended for inclusion in this project were unable to tolerate the dosage of quinidine and dropped from the study. The exact frequency cannot of course be stated but in several cases there appeared to be true, severe cinchonism. Of the 44 patients completing the study, 11 had symptoms suggestive of cinchonism at the start and may be excluded from this consideration. Of the remaining 33, 28 developed tinnitus, deafness, vertigo, visual difficulties, or gastrointestinal disorders during drug administration. Once again, these complaints could be evaluated properly only by the "blind" comparison with the placebo. Of these 28 individuals, 18 had symptoms on both quinidine and placebo, none on placebo alone, and 10 on quinidine alone. These last 10 cases, remaining after exclusion of those patients unable to complete the study, those with possible cinchonism-like symptoms at the start, and those developing symptoms alike on the alkaloid and lactose, gave a minimum incidence of apparent cinchonism of some 23%.

EFFECTS OF QUININE. After completion of the quinidine-placebo study and at least 2 additional weeks without treatment, 18 patients who still manifested hypertension were carried through similar 4-week courses of quinine and placebo. The "original" blood pressure was that of the last

non-treatment week before this therapy was instituted. Thus the starting point for this phase of the project was the pressure after some 2½ months of study. In 10 cases quinine was given first and in 8 placebo first. Since results were similar to those obtained with quinidine, they may be presented only briefly.

TABLE 3.—EFFECT OF QUINIDINE AND PLACEBO ON SYMPTOMATOLOGY OF 44 PATIENTS

	During Quinidine therapy		*During Placebo therapy*	
	Better %	*Worse* %	*Better* %	*Worse* %
Over-all Impression				
Patient's opinion of week	41	23	38	23
Doctor's opinion of week	28	17	26	13
Specific Symptoms				
Headache	37	28	40	24
Palpitation	40	14	42	12
Weakness	21	41	27	35
Chest Pain	27	16	29	23
Nervousness	27	15	30	10
Dizziness	25	32	25	30

Figures expressed in per cent of total weekly reports in each category.
Factors considered unchanged are omitted from tabulation.

TABLE 4.—EFFECT OF QUININE AND PLACEBO ON MEAN BLOOD PRESSURE

		By order of administration	
	Total	*Quinine first*	*Quinine second*
Number of Patients	18	10	8
Systolic Pressure			
Original[1]	180 ± 24	181 ± 18	180 ± 31
Quinine[2]	182 ± 25	181 ± 20	182 ± 31
Placebo[2]	181 ± 21	184 ± 14	178 ± 27
Diastolic Pressure			
Original[1]	108 ± 15	106 ± 15	111 ± 15
Quinine[2]	108 ± 17	107 ± 17	110 ± 22
Placebo[2]	109 ± 15	107 ± 14	111 ± 17

Values expressed as mean ± standard deviation in mm. of mercury.
[1] from 6 readings on each patient at last visit before quinine or placebo instituted.
[2] from 18 readings on each patient during last 3 weeks while receiving drugs.

Blood Pressure. There was no important change in the blood pressure during this second portion of the study. The mean pressure for the group was 180/108 at the start, 182/108 while on quinine, and 181/109 during administration of placebo. (Table 4.) Differences between the average pressure under quinine therapy and both the original value and the readings obtained while on placebo were insignificant. By individual patients, quinine lowered the systolic pressure more than did lactose in 3 instances and the diastolic in 4 cases. However, the placebo had a greater effect on the systolic tension of 5 and the diastolic of 4. These minor variations were undoubtedly due to chance.

Symptomatology. Quinine was of less benefit to symptoms than was the placebo. Only 30% of the weekly intervals were considered by the patients

to have been improved by the alkaloid while 40% were better on the placebo. In the opinion of the physicians seeing the patients, 29% of the placebo-weeks showed improvement and only 24% of the quinine-weeks. On the average, 9% of the symptoms were better when quinine was taken while 15% showed improvement on lactose.

Toxicity. Cinchonism was encountered in many patients who had tolerated quinidine but were unable to complete the course with quinine. Of the 18 finishing this course, 10 either had had no symptoms of cinchonism during quinidine treatment or previous toxic symptoms had disappeared. All of these reported toxic effects in the quinine-placebo phase, 5 on both quinine and placebo, none on placebo alone, and 5 on quinine alone. Thus evident cinchonism developed in at least ¼ of the group.

Discussion. The results obtained yield information on 2 points; the effect of cinchona alkaloids on human hypertension and the problems inherent in evaluation of hypotensive agents. The former subject may be considered briefly. Certainly neither quinidine nor quinine was of the slightest specific benefit to the well-being of the patients, whether one considers the patients' opinions of their general health, the physicians' evaluation of the clinical course, or the status of individual symptoms. In each case, the apparent benefits induced by the alkaloids were equalled by the lactose placebo. Similar comparison with the results of placebo therapy indicated that quinine and quinidine were also without particular effect on the arterial pressure. It may be that the failure to confirm the previously reported vasodepressor actions of the alkaloids was due to the smaller amounts administered in this investigation. The group of Ferrer[5], for instance, used a single dose of 0.9 gm. of quinidine. However, the present study was designed to evaluate the prolonged use of the drugs. The dosage system used, 0.3 gm. 3 times daily, was found commonly to produce undesirable side-actions and could not be tolerated by many patients, particularly when quinine was administered. An attempt was made to repeat the test of quinidine, giving 0.6 gm. at a dose, but this proved so toxic that the procedure was abandoned promptly. It can be assumed that the amounts prescribed were as large as could be tolerated by most patients on a long-term basis.

Of greater import were the observations related to evaluation of hypotensive drugs. It should be emphasized that this experiment was performed on a group of patients with hypertension of long standing. All had received various forms of treatment according to usual clinic procedures for long periods, in some cases for several years. The multiple previous determinations of the blood pressure were in good agreement with the initial readings obtained in this test, indicating that the starting point of the study was a representative one. Certainly the group could safely be considered as "stabilized" hypertensives. Yet the mere institution of a new treatment situation, with more time and concern devoted to each patient and the dispensation of a new drug, produced a lowering of the blood pressure which was often of a dramatic nature and which continued for some months. Ayman[2] described these effects of treatment *per se* in 1931 but even today the problem is not fully appreciated. Ordinarily the only controls in a study of anti-hypertensive therapy are statistical analyses. Such methods have been used herein and have proved useful, but only as a check upon the random variability of the blood pressure. It must be reiterated that hypertension varies not only by chance but additionally in response to

a definite factor, the nonspecific effects of treatment. Mathematical interpretations alone cannot control this factor and statistics are no substitute for a properly designed experiment. The procedure used in the present experiment appears to control the nonspecific effects of therapy and to permit accurate evaluation of drugs. Certainly if this study had been performed in the usual manner, (with determination of the "control" status of the patients, administration of the test drug alone under "non-blind" conditions, and statistical analysis of the results) completely erroneous conclusions as to the value of quinidine would have been reached.

The "double-blind" principle also appears to be essential. The importance of using a placebo, while keeping the patient ignorant of the fact, is obvious from inspection of the data. Probably even more important is the need to keep the physician uninformed of the identity of the substance administered. We are convinced that the subconscious bias of investigators, either for or against a proposed measure, will be manifest in the results obtained. This view has some support from other quarters. Ayman[1] demonstrated that placebos effectively controlled symptoms in hypertension when administered with deliberate enthusiasm. Jonas[10] has shown how a physician, without words but simply by facial expressions, can alter the blood pressure at will. It is essential then to conduct studies in hypertension so that the attitudes of the physicians cannot affect the data and conclusions; the "double-blind" technique is suggested for this purpose.

The unreliability of subjective factors in hypertension, as a guide to results of treatment, was also demonstrated. The symptoms bore no visible connection to any other factor. There was no relationship between the height of the blood pressure and the severity of symptoms in any individual and none between symptomatic improvement and changes in the pressure. Patients often reported themselves improved by treatment yet were unable to distinguish between the effects of the alkaloids and the placebos and frequently contradicted themselves on detailed questioning. Attending physicians, possibly with caution induced by the "double-blind" nature of the experiment and the necessity of forming an opinion each week before determining the blood pressure, came to conclusions concerning the patients' progress which often were at variance with the condition of the pressure and the opinions of the patients themselves. It can only be concluded that opinions on subjective factors, whether expressed by the patients or the physicians, are meaningless. Indeed it would seem that the symptoms of hypertension have little if any connection with the hypertension itself. This opinion is supported by the recent report of Stewart[11] who found that the incidence of headache, in particular, was related not to the presence of hypertension but to the patient's awareness of an elevated pressure. He concluded that in most instances this symptom represents a manifestation of anxiety. Similarly, Droller, Pemberton and Roseman[4] found that the presence or absence of hypertension did not influence the frequency of "hypertensive" symptoms in elderly patients.

Summary. The "double-blind" principle for analysis of drug effects has been adapted to a study of therapy in hypertension. The efficacy of the cinchona alkaloids has been analyzed by this procedure.

Quinidine produced apparently dramatic effects on the blood pressure and symptomatology of hypertensive patients. However, the results were du-

plicated by use of a lactose placebo and it was concluded that quinidine is of no value in hypertension. Similarly, quinine was shown to be without specific effects.

The observations have been discussed in relation to the difficulty in evaluation of therapy for hypertension. The chief points made were that it is possible to lower the blood pressure and improve the symptomatology even in "stabilized" hypertensives, that subjective opinions both of patients and doctors are valueless in evaluation of therapy, and that statistical analysis alone is not an adequate control of the experiment. It is considered that the "double-blind" characteristic should be an integral part of any studies on hypertension.

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ON THE RELATIONSHIP OF URINARY COPPER EXCRETION TO THE AMINOACIDURIA IN WILSON'S DISEASE (HEPATOLENTICULAR DEGENERATION)

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In recent years the study of the metabolic factors operative in the pathogenesis of hepatolenticular degeneration have followed two main lines of approach, each being based on observations seemingly unrelated in their nature, but both having definite specificity for this disease in their occurrence. The persistent and often massive aminoaciduria first described in this Journal[21], and since then amply confirmed[2,4,5,6,11,15,16,19,20,21,23], has emerged as the single most constant metabolic feature of the disease, being present as a familial stigma, and occurring in asymptomatic siblings in a family where one or more members are affected with the disease[12,22-24]. The characteristic pattern in its occurrence and the lack of correlation between the aminoaciduria and the degree of hepatic involvement has been emphasized previously[22], and has received due attention in a recent comprehensive review on the subject[1].

The second path toward elucidation of the underlying metabolic factors that eventually produce the disease entity has followed the observations made some two decades ago of an increased amount of copper deposition in the brain and liver[10,13] in these patients. Although heavy metal deposition had been considered as a possible etiologic agent at an early stage of our recognition of the disease entity, arguments to the contrary had prevailed until recently, when the original observations of Haurowitz[10] were confirmed and enlarged upon by Mandelbrote and his associates[14], Cummings[3], and others[9,17]. These observations, in turn, prompted therapeutic trials with BAL (2, 3, di-mercapto-propanol) injections to patients in an effort to remove the accumulated copper from the tissues. Such attempts initiated by Denny-Brown and his associates[5] in the United States, and reported by Cummings[4] in England, provided patients with temporary remissions and sometimes with considerable amelioration of symptoms. It became evident, however, that the removal of copper *per se* could not influence the inherent metabolic defect, and was probably only effective in liberating temporarily, for functional purposes, metal-bound cytoplasmic constituents and enzymes. Treatment with BAL did not alter the aminoaciduria[4,5] of these patients, nor was clinical amelioration of symptoms accompanied by decrease in the aminoaciduria[4,5,23]. These observations,

together with the findings that asymptomatic siblings of patients with Wilson's disease showed no cupruria nor increased copper excretion on treatment with BAL, although demonstrating persistent aminoaciduria, prompted the suggestion that two distinct, but probably mutually complementary factors were responsible for the nosologic entity designated as hepatolenticular degeneration[1,22].

The aminoaciduria was considered to be the primary defect since it appears to be present in the asymptomatic stages of the disease, occurs in asymptomatic siblings of affected patients, is found to be independent of the degree of hepatic involvement, and is unrelated to the severity of neurologic symptoms. A second factor, possibly related to the primary defect causing the aminoaciduria, was postulated[1,5,22] to account for the involvement of the nervous system. The disturbance manifested by excessive copper accumulation in the tissues was incriminated as representing this second factor[1,4,5,19], although no *modus operandi* could be assigned to it. Attempts to establish a relationship between the cupruria and aminoaciduria became clearly indicated as a preliminary step in evaluating the significance of copper in the pathogenesis of hepatolenticular degeneration. Such an attempt was forthcoming in the interesting work of Mathews, Milne, and Bell[15], who showed that the urinary excretion of copper in their 2 patients could be increased by the ingestion of large doses of glycine and alanine. It was inferred that the copper was probably excreted in the form of a copper-chelate in which the amino acid forms a non-ionizable complex with the copper. Thus, the metal, though normally not diffusible because of binding with serum proteins, could pass into the glomerular filtrate in the chelated form. The occurrence of just such a state, however, could not be shown. The results of these investigators suggested the conclusion that the abnormally high excretion of copper in the urine in the absence of proteinuria occurs only when there are both increased copper in the tissues and abnormal aminoaciduria, this combination being almost specific for Wilson's disease. Because of the lack of information on simultaneous determinations of copper in tissues, blood serum, and urine, the strange paradox which hepatolenticular degeneration is reported to present in terms of combining an abnormally elevated copper retention in tissues with excessive elimination of the metal in the urine presents an incongruity difficult to explain except by postulating a greatly increased absorption of copper from the intestinal tract in this disease[15]. Evidence for this is lacking, and the paradox may well be resolved by the finding that copper retention in tissues on one hand and cupruria on the other represent different phases of the disease process, especially since asymptomatic siblings of patients having Wilson's disease have been found to have the characteristic aminoaciduria, without exhibiting cupruria. Nevertheless, establishing the relationship of the aminoaciduria to increased copper excretion, in patients who do exhibit the latter in the resting state, would be an important step in the elucidation of the chemical pathogenesis of the disease beyond the suggestion of a lowered renal threshold for both substances. This becomes especially important in view of the specific peptiduria represented by the excretion of oligopeptides with dicarboxylic acids as terminal residues recently described in this disease[22] and the known affinity that such peptides possess toward formation of stable complexes with copper. That the mechanism of enrichment of tissues in copper in hepatolenticular degeneration may reside in

the formation of stable complexes between copper and tissue peptides with terminal dicarboxylic acid residues has been suggested by Brick[1], assuming that the specific peptiduria reflects a disturbance in tissue proteolytic enzymes, resulting in the accumulation of abnormal peptide residues in the cells of organs that eventually do show increased amounts of copper deposition.

The present study had its origin in the observation that the specific peptides previously described did not appear on the chromatograms of fractionated urine samples of a classical case of Wilson's disease in spite of the presence of gross aminoaciduria. Investigation of the possible reason for our failure to detect such peptides with ninhydrin spraying culminated in the finding that these peptides were present as copper-chelate complexes in the urine samples examined, and hence would not be expected to react with ninhydrin when partitioned on paper chromatograms. However, the release of copper could be effected by acid treatment, and the peptides could then be identified with ninhydrin. This observation provided a clue to the mechanism of cupruria in cases accompanied by aminoaciduria in Wilson's disease, and was further investigated by development of a technique of fractionation of the urine of such patients for isolation of the peptide-copper complexes. It could be shown that the bulk of the urinary copper excreted by a patient with Wilson's disease appeared in the urine as a chelate with dicarboxylic amino acid peptides.

Report of Case. A 14-year-old boy (patient of Dr. John A. Davies) was admitted to the Boston Children's Hospital in October, 1951, with a history of progressive slurring of speech and incoordination of movements of arms and legs of 2 years' duration. Onset of symptoms had been marked by "giggliness" and emotional incontinence. There were no headaches, blurring of vision, or sensory disturbances at any time. Swallowing of food had become slightly difficult at the time of admission. Tremor of the extremities was denied. The past history was non-contributory. There had been no severe illness with a prolonged febrile episode. The patient was an only child. Jaundice and convulsive seizures were denied. The family history was highly suggestive in that one paternal and two maternal cousins had died of "degenerative brain diseases" of unkown types. More information on this aspect of the history was unavailable.

Physical examination disclosed a well-nourished, well-developed boy in no physical discomfort. Marked euphoria and a silly, fixed grin were evident. Examination of the cornea disclosed bilateral Kayser-Fleisher rings of classical type. The liver was just palpable at the right costal margin, presenting a smooth, nontender edge. There were no spider angiomas, nor evidences of portal hypertension. Neurologic examination disclosed no abnormality of cranial nerves. Nystagmus was absent. Eye grounds were normal. The lips were kept parted constantly, and there was persistent drooling of saliva. Speech was slurred with an explosive quality at the terminal syllable. There was palatal tremor without paralysis. Motor power was good throughout the body. Tendon reflexes were bilaterally equal and exaggerated. There was no ankle clonus. Bilateral Babinski signs were easily elicited. There was marked motor incoordination, affecting the upper extremities more severely than the lower. There was no tremor at rest. A severe cerebellar type of ataxia with a flapping-action tremor was evident on finger-to-nose tests, bilaterally. The heel-to-shin tests were poorly performed. The gait was ataxic, but Romberg's sign was absent. Mild rigidity was observed on passive motion, involving the arm and leg on the right side. Titubation of the trunk was evident. The sensorium was intact in all modalities.

Examination of the blood and routine laboratory tests were entirely normal. The urine was constantly acid with a specific gravity varying between 1.013 and 1.036. No reducing substances were detected on repeated examination. Prothrombin time was 13.1 seconds (control, 14.2 seconds). Bromsulfonphthalein tests (5 mg./kg.) showed 3.2% residual dye after 30 minutes and 2.4% dye after 45 minutes. The thymol turbidity was 1.0; thymol flocculation, 0, and cephalin flocculation was reported as one plus. On

repetition 3 months later, the results of liver function tests were essentially the same. Spinal fluid examination was entirely negative. On two separate occasions, estimation of the serum alpha amino nitrogen gave values of 4.55 and 4.58 mg. per 100 cc.

The patient was seen by Dr. Denny-Brown and BAL therapy was started on his recommendation. Intramuscular injections of 1 cc. of BAL (10% solution in peanut oil) twice daily for 20 days, followed by a rest interval of one month, was the schedule used. Three such courses were given. The urine was collected just prior to each BAL course for the studies reported in the following sections. The motor incoordination appeared greatly improved after this therapy, drooling and slurring of speech were decreased, and the patient was able to attend a special school. However, 8 months after the initial admission, the liver was palpable 2 fingerbreadths below the right costal margin, although the liver function tests were still normal. Permission for biopsy of the liver was refused.

STUDIES ON THE URINE. The urine was collected as pooled 24-hour samples during periods when the patient was not receiving BAL. Toluene was used as a preservative in each instance. The total volume of each sample was measured, and the samples were allowed to stand at 0° C. until analyses could be carried out. All samples studied were free of protein and reducing substances. Appropriate aliquots were removed for determination of total alpha amino nitrogen by formol titration. Smaller aliquots were used for one-dimensional and two-dimensional paper chromatography, using phenol-water as the first and butanol-acetic acid-water (50:10:40) or collidine-water, or butanol-ethanol-pyridine (60:20:20) as the second solvent system.

The patient was found to have persistent and massive aminoaciduria (Table 1). Chromatography showed this to be generalized in nature with a preponderance of threonine, arginine, asparagine, and alanine. On one occasion 735 mg. of tyrosine could be crystallized out directly, by allowing the urine (pH 6.0) to stand 24 hours at 2.0° C. This aminoaciduria was accompanied by cupruria that showed considerable variation and appeared to bear no direct relationship to the magnitude of the aminoaciduria (Table 1). Copper was measured by the method of Eden and Green[7]. Preliminary semiquantitative estimates of the copper content were made by wet digestion of 15 cc. of urine with concentrated HCl followed by fuming nitric acid digestion. The residue was evaporated to a solid crystalline mass, dissolved in water, and neutralized with alkali. Different amounts of known aliquots were then used for the detection of copper with rubeanic acid* by spot test, according to Feigl[8]. Samples that had too low copper contents were not used in the fractionation procedure described in the following section.

FRACTIONATION OF URINARY PEPTIDES. The procedure described previously[22] for the fractionation of urinary peptides having dicarboxylic amino acid terminal residues was slightly modified for the separation of peptide-copper chelates. The urine was acidified with N-HCl to pH 5.5 and a 10% $BaCl_2$ solution was added dropwise. The ensuing heavy white precipitate was removed by filtration and discarded, and more barium chloride was added until no more precipitate formed. The urine was filtered, and the clear supernatant was tested with a few more drops of $BaCl_2$ solution to ensure the removal of all precipitable material at acid pH. To the supernatant a saturated solution of barium hydroxide was next added dropwise until the reaction mixture was brought to pH 7.8. The heavy white flocculate which ensued was removed by centrifugation (Fraction Ba I). To the combined clear supernatants an equal volume of 95% ethanol was added, and the mixture was allowed to stand in the cold room at 2° C. overnight. The ensuing precipitate was collected by centrifugation and designated Fraction Ba II. To the supernatant from this fraction more ethanol was added in an amount corresponding to the original volume of urine. The ensuing precipitate was again collected (Fraction Ba III) and the supernatant was again treated with more ethanol (equal in amount to the original volume of the untreated urine) to yield Fraction Ba IV. Fraction Ba V was obtained from the supernatant in like manner by progressively increasing the ethanol concentration. Fractions Ba VI to Ba IX were negligible in amount (ca 10 mg./liter of urine) and were discarded after ascertaining that they contained no copper. Ba X contained the bulk of the urinary aspartic and glutamic acid in the form of the barium salt, as ex-

*Rubeanic acid (dithio-oxamide, $H_2N-\overset{S}{\overset{\|}{C}}-\overset{S}{\overset{\|}{C}}-NH_2$) is a sensitive reagent for copper, yielding a brown-black precipitate in alkaline or slightly acid medium, the concentration limit being about 1:2,500,000. The reagent was added as a 1% soln. in 50% ethanol.

pected according to the principle of Foreman precipitation of these amino acids.

Barium Fractions I, II, III, IV, V, and X, obtained in the foregoing manner were then treated with 0.5N H_2SO_4 with special care so that at no time did the pH go below 7.0. Each fraction was placed in a water bath at 40° C. for about 30 minutes, and the precipitated $BaSO_4$ removed by centrifugation. The barium-free supernatants were then made up to 25 cc. in volume with water, and suitable aliquots (A) were removed for copper estimations[7]. Other aliquots (B) were taken for chromatographic analysis. A third aliquot (C), 10 cc. in amount, was taken to dryness *in vacuo* and treated with 2 cc. of 6N HCl for 20 minutes at 40° C. to liberate the copper. The acid solutions were brought to pH 6.0 by addition of dilute alkali, and the copper ions were quantitatively precipitated by addition of 1.0 cc. of a 1% solution of rubeanic acid.* The precipitate was removed by centrifugation, and the supernatant was reduced to 1.0 cc. in volume *in vacuo*.

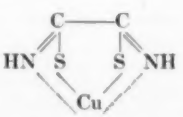
Paper chromatography of aliquots (B), in phenol-water systems, showed no ninhydrin-reacting spots. Accordingly, other strips were sprayed with 0.5N HCl, and the paper was heated in an oven at 120° C. for 10 minutes. After this period, a 1% ethanolic solution of rubeanic acid was evenly sprayed on, and the paper was allowed to stand in air. Within a few minutes, dark brown spots appeared which could be intensified by placing the paper for 5 minutes in an oven at 110° C. The spots had an R_F of 0.20 to 0.38, and their size varied in direct proportion to the amount of material placed on the starting line of the chromatogram. Chromatograms which were first treated with 0.5N HCl, heated, and subsequently treated with 0.2% ninhydrin in n-butanol showed strong purple areas corresponding exactly to the areas that reacted with rubeanic acid to give a black area. This was taken as indicating that the copper in these fractions is present as a complex with the peptides, this complex being broken by acid treatment, thus allowing the peptides to react with ninhydrin. Other chromatograms to which 0.05 to 0.10 μg. of copper as copper chloride were added as controls showed no circumscribed areas when sprayed and developed with rubeanic acid, but gave streaks having their origin at the starting point.

Chromatograms of aliquots (C) showed no dark areas on treatment with rubeanic acid, indicating prior removal of all the copper. Treatment with ninhydrin revealed purple-red staining spots with R_F of 0.24 to 0.42. Accordingly, larger aliquots of (C) were placed as a line on large (14 by 18 inches) sheets of Whatman No. 1 filter paper and chromatographed with phenol-water. A guide strip was treated with ninhydrin, and the corresponding area was cut out of the large sheet. The material was eluted overnight with water to yield a final volume of 1.4 cc. This material was divided into two equal portions,

TABLE 1.—AMOUNTS OF AMINO NITROGEN AND COPPER EXCRETED BY OUR PATIENT

Sample	*24-Hour urine volume, cc.*	*Total alpha amino nitrogen per 24 hrs., mg.*	*Total urinary copper, μg.*	*Peptide amino nitrogen as % of total amino nitrogen*
1	1570	1894	317	15.2
2	1680	1120	640	20.2
3	1120	1160	Traces	15.4
4	1770	1445	17	16.8
5	860	1760	217	17.4
6	1580	876	495	16.8
7	1500	1120	Traces	8.0
8	1675	982	24	11.0

*The black precipitate that is thus formed is believed to consist of the inner complex salt of the di-imido form of rubeanic acid:



one of which was hydrolyzed with 6N HCl in sealed tubes at 110° C. for 6 hours for chromatographic analysis of the amino acid constituents of the peptide, and the other treated with 2-4-dinitrofluorobenzene to label the terminal amino acid residue[18]. Identification of the ninhydrin-reacting substances as oligopeptides having aspartic or glutamic acid terminal residues (with the amino group of the dicarboxylic acid free) was effected by the method described previously[22].

In some instances in which the content of amino acids in the urine was extremely high (more than 1.0 mg. amino-nitrogen/cc.), the barium-ethanol fractionation also led to the precipitation of amino acid-barium salts together with the peptides. This was most pronounced in Fractions Ba I, Ba II, and Ba III. After removal of the barium as described, the amino acids could be separated from the peptides by taking the supernatant almost to dryness *in vacuo* and extracting the brownish magma exhaustively with hot n-butanol (60° C.). The peptides were recovered in the butanol-insoluble residue and studied as has been described.

The distribution of the urinary copper with respect to the various fractions described is given in Table 2. It is evident that the bulk of the urinary copper is found in the fractions that contain the peptides excreted in Wilson's disease. No significant amount of the metal could be detected in those fractions containing the bulk of the free amino acids. Examination of the fractions obtained from six 24-hour specimens showed the bulk of the urinary copper to be present in those fractions designated as Ba I and Ba II, while chromatography indicated unequivocally that the copper migrated with the urinary oligopeptides present in these fractions.

No complex formation with amino acids or peptides could be shown, by chromatography or fractionation, when known amounts of copper (100 to 1000 μg. of copper/liter of urine) were added to normal male urine or to the urine of our patient with Wilson's disease.

Discussion. From the data presented, it appears that the copper found in the urine in Wilson's disease is present mainly as a complex with urinary peptides of a specific structure, that is, those having dicarboxylic amino acids as terminal residues. This would constitute strong evidence for the concept that the copper ultimately recovered in the urine passes from the blood into the glomerular filtrate in the form of the copper-peptide chelate. Although the diffusion of serum copper into the glomerular filtrate in normal subjects cannot be inflexibly denied at the present time, the bulk of the evidence, as summarized by Mathews and his associates[15], would tend to support the *in vitro* work that shows all the serum copper to be present in undialyzable form, being bound to serum proteins. It also seems unlikely that the urinary copper is derived from the filtration of

TABLE 2.—THE ASSOCIATION OF URINARY PEPTIDES AND COPPER

Fractions	*Copper, μg.*	*Chromatogram developed with rubeanic acid*	*Chromatogram of samples freed of copper (aliquots C) developed with ninhydrin*	*Copper recovered in fractions as % of total copper excreted in urine (680 μg.)*
Ba I	320	Single large area +++	Single large area (peptide) R_F=0.24 to 0.42 (phenol-water)	47.0
Ba II	270	+++	Single large area R_F=0.20 to 0.40	39.8
Ba III	17	+	Peptide (faint), plus glutamic acid, aspartic acid, and lysine	2.5
Ba IV–IX	None	None	Traces of threonine, serine, leucine, and valine	None
Ba X	None	None	Aspartic acid and glutamic acid	None
Residual urine	Traces	None	14 amino acids, but no glutamic or aspartic acids	—

amino acid-copper complexes from serum, since the formation of such complexes cannot be shown to take place even with enormous plasma-free amino acid concentrations produced artificially[15,23], the plasma-bound copper having a much higher affinity for the plasma proteins than for free amino acids. This is supported by the observation that the magnitude of the aminoaciduria bears no relationship to the cupruria in the resting state, as was also observed in the present study. One is thus directed to the conclusion that the urinary copper-chelates are derived directly from copper-peptide complexes released from tissue sources and are not formed *de novo* in the plasma of patients with Wilson's disease. Whether the peptide moieties of these copper-chelates exist as such in tissue or whether they are formed in the course of faulty proteolytic activity leading to incomplete hydrolytic cleavage of tissue proteins is not germane to the present problem, although it is undoubtedly crucial in the eventual formulation of the pathogenesis of the disease. However, the presence of copper-peptide complexes in the urine in this disease strongly suggests that the formation of such copper complexes with larger peptide residues in tissues may explain the fixation of this metal in organs like liver and brain. In this sense, the deposition of copper would be the manifestation of a process corollary to the original defect in the synthesis and breakdown of tissue proteins resulting in urinary excretion of oligopeptides of specific nature, and aminoaciduria. The existence of such a state was proposed recently by Brick[1]. The finding of an increase in the cupruria as a result of artificially induced elevations in the serum glycine and alanine levels in these patients[15] constitutes insufficient basis for the assumption that the copper is excreted as an amino acid-copper chelate in hepatolenticular degeneration. In none of our studies could the existence of such chelates be shown in the urine of our patient, in spite of pronounced aminoaciduria. An alternate explanation for the observations of Mathews and his associates can be sought in the factors responsible for the aminoaciduria itself. Thus, if the aminoaciduria is due to competition for resorption at the tubular level between amino acids and oligopeptides, such competition resulting in the preferential resorption of oligopeptides (with their partial elimination in the urine) and exclusion of amino acids (resulting in gross aminoaciduria), as suggested previously by us[22], sudden increases in the concentration of a particular amino acid in the glomerular filtrate, as induced by Mathews and his associates[15] would upset the equilibrium for competitive resorption precariously maintained, resulting in decreased tubular absorption of oligopeptides. Therefore, if the oligopeptides are present at times as the copper complexes (as shown in this study) in the glomerular filtrate, an increase in the aminoaciduria induced by the ingestion of glycine and alanine would also result in an increase in the excretion of copper. In contrast, no such rise in urinary copper was found in 4 normal subjects in spite of a comparable rise in serum and urinary amino-nitrogen levels in the studies of Mathews and his associates[15]. This observation is perfectly reconcilable with the concept that the "renal aminoaciduria" of Wilson's disease is different in nature from the "renal threshold overflow aminoaciduria" induced in the normal subject, and that the "renal threshold" denotes qualitatively quite different processes in each of the two states. Since the "lowered renal threshold" for amino acids in hepatolenticular degeneration appears to be the manifestation of the competition of two classes of substances for resorption at the tubular level, overloading of the glomerular filtrate with amino acids

can result only in upsetting the established equilibrium in the competitive reabsorptive process by increasing the concentration gradient of amino acids presented to the tubular absorptive mechanism. Change in the composition of the tubular fluid in terms of one of the competing constituents can be expected to give rise to a change in the absorption of the other. These considerations serve to underscore the necessity for simultaneous studies on tissue, plasma, and urine in hepatolenticular degeneration for clarification of the nature of the aminoaciduria and cupruria on one hand and the reported increased tissue deposition of copper on the other. A clearer understanding of these factors would remove the main obstacle to the formulation of the true nature of this interesting disease. The studies reported from different parts of the world in recent years have presented so far a detailed scrutiny of individual facets of the entity, without offering a clear picture of the whole.

Summary. A case of hepatolenticular degeneration manifesting persistent massive aminoaciduria accompanied by increased urinary copper excretion was studied with respect to the relationship of the aminoaciduria to the cupruria. The urinary copper was isolated in the form of a complex with oligopeptides of a specific nature previously described. The daily output of copper bore no relationship to the aminoaciduria itself. It is suggested that the oligopeptides-copper chelate represents the form in which copper passes from plasma into the glomerular filtrate, and that the urinary excretion of the metal is a result of the competition, for tubular resorption, between the oligopeptide complex and the amino acids present in high concentration in the tubular fluid.

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THE EFFECT OF NOREPINEPHRINE AND EPINEPHRINE ON RENAL HEMODYNAMICS*

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Altered renal hemodynamics associated with changes in blood pressure have been an interesting problem to the physiologist as well as the clinician. Renal ischemia resulting from severe hypotension has supposedly led to the patients demise as a direct result of renal excretory failure despite the fact that the original cause of the hypotension was eventually corrected. Renal vasoconstriction under these circumstances may be due to sympathetic nervous system activity (direct effect) and the release of vasopressor substances such as norepinephrine and epinephrine (indirect effect). The first and foremost purpose of the present report was to study mechanisms by which norepinephrine or epinephrine produced acute alterations in renal function and to evaluate differences in response between the human kidney and the canine kidney previously studied in a similar fashion in this laboratory. This seemed indicated since differences of opinion throughout the literature on the hemodynamic response to norepinephrine and other vasopressor agents may actually be a species difference. Certainly, differences in renal hemodynamic response to epinephrine[1,5,7,9,10] seems well documented. Therefore, this agent was also evaluated in the current study for comparative purposes. Trueta[10] has presented evidence in animals which suggests that epinephrine constricted the more distal portions of the renal arteries so that cortical glomeruli and their associated tubules were functionally removed from the circulation. At the same time juxtamedullary glomeruli and their associated vascular channels were said to have become more active so that in effect there was a renal arterio-venous shunt, although it was not a direct one in the usual sense of the term. Previous studies in dogs[5] in this laboratory using similar experimental techniques to those in the present study have shown a reduction in the number of active nephrons following administration of both norepinephrine and epinephrine, but have not shown evidence of a renal vascular shunt as measured by the renal arterio-venous oxygen difference. Contrariwise, after the administration of epinephrine to human subjects Smith[9] was unable to demonstrate a decrease in the number of active nephrons, since the amount of functioning tubular tissue as measured by

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the maximum tubular transport of glucose (TmG) and diodrast (TmD), remained constant. It was of interest to note that in the current study there was a similar difference in response between the human and canine kidney following administration of norepinephrine, which is a much more potent vasoconstricting agent.

In the treatment of severe hypotensive states which occur despite normal blood volume, norepinephrine has frequently proven to be of great value in raising the blood pressure and thus shortening the duration of the hypotension[8]. Increasing the blood pressure in this manner is frequently associated with improved renal function as estimated by urinary output, a seeming paradox from the vasoconstrictive effect of norepinephrine. Therefore, as a second part of the study it seemed desirable to observe the response of administering norepinephrine to patients in the hypotensive state. Consequently, it was given to normal control subjects and hypertensive patients, in whom the blood pressure had previously been lowered with hexamethonium. The purpose of this procedure was to determine if there was any difference in renal hemodynamic effect between raising the blood pressure from a reduced level to one approximately normal for the patient and raising the blood pressure above the patients usual level to hypertensive ranges as was done in the normotensive group (Patients Nor 1 to Nor 24) not given hexamethonium.

Methods. Twenty-eight patients received norepinephrine or epinephrine alone. All had blood pressures within the normotensive range, (MBP < 110 mm. Hg) except for 4 patients who had borderline hypertension at the time of the study (Nor 17, 19, 23 and Epi 6). One patient had severe renal impairment as the result of chronic pyelonephritis, and was studied on 3 separate occasions (Nor 22, 23, 24). After the blood pressure had been lowered by the administration of hexamethonium, norepinephrine was given to an additional 8 patients with hypertension and varying degrees of renal disease, and to 7 normotensive patients (Nor 25 to 39, Table 4). Three patients in this group were on oral hexamethonium therapy, and 12 received the drug intravenously (continuous infusion) on the day of the study. After the norepinephrine infusion was started hexamethonium was given at the same rate as during the previous hypotensive period in order to maintain an equivalent degree of ganglionic blockade.

Renal clearances were performed by standard techniques[3] and the methods have been previously described[6]. Endogenous creatinine was used for estimating creatinine clearance. The clearances were not corrected to standard surface area in the present study. With only minor changes the techniques used were the same as those employed in the previous study in dogs[5]. Mean blood pressure was arbitrarily taken to be one-third of the pulse pressure plus the diastolic pressure, with the exception of those studies in which it was measured directly by means of an intra-arterial needle connected to a mercury manometer. After 2 to 4 successive 10-minute control periods, an infusion containing 4 mg. of l-norepinephrine* or epinephrine* per liter was started. An attempt was made to adjust the infusion rate so that the systolic blood pressure was increased 30 to 50% above the control values. When the blood pressure had again stabilized at a level considered safe for the patient, renal clearances were again measured for 2 to 4 consecutive 10-minute periods. In the patients who received hexamethonium, norepinephrine was begun only after the hypotensive response had been well stabilized.

Results. The effects of norepinephrine on mean blood pressure and renal hemodynamics (normotensive subjects) are presented in Tables 1A and 1B. Infusion of the drug in an average dose of 0.321 micrograms/kg./min. increased the average mean blood pressure to 136% of the control value. As a result of a marked increase in renal vascular resistance, renal plasma flow (RPF) was depressed to 60% of the control value, however, glomerular filtration rate (GFR) was usually maintained near the control level.

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TABLE 1A.—EFFECT OF NOREPHINEPHRINE ON GLOMERULAR FILTRATION RATE AND RENAL PLASMA FLOW

	UV			*GFRman*			*GFRcr*			*RPF*			*FFcr*		
Patient	*C*	*NE*	*%*	*C*	*NE*	*%*	*C*	*NE*	*%*	*C*	*NE*	*%*	*C*	*NE*	*%*
Nor. 1	4.2	15.2	362	125	110	88	152	142	93	985	517	52	.154	.275	179
Nor. 2	4.1	11.5	280	104	101	97	117	99	85	667	439	66	.175	.226	129
Nor. 3	17.4	9.1	52	...	...	...	104	88	85	651	394	61	.160	.223	139
Nor. 4	11.5	12.1	105	117	121	103	152	157	103	905	489	54	.168	.321	191
Nor. 5	5.8	4.1	71	...	...	...	98	95	97	432	312	72	.227	.304	134
Nor. 6	5.9	1.6	27	...	...	...	122	133	109	758	453	60	.161	.294	183
Nor. 7	6.0	5.7	95	116	113	97	132	136	103	639	345	54	.207	.394	190
Nor. 8	3.9	3.3	85	...	...	...	120	142	118	656	524	80	.183	.271	148
Nor. 9	13.7	8.2	60	78	65	83	81	71	88	664	282	42	.122	.252	207
Mean	8.1	7.9	126	108	102	94	120	118	98	706	417	60	.173	.284	167

Key to Abbreviations

UV = Urine volume: cc./min.
GFRman = Mannitol clearance: Glomerular filtration rate: cc./min.
GFRcr = Creatinine clearance: Glomerular filtration rate: cc./min.
RPF = p-aminohippurate clearance: Total renal plasma flow: cc./min.
C = Average of control values

$$\text{FFcr} = \text{Filtration fraction: } \frac{\text{GFRcr}}{\text{RPF}}$$

NE = Average values during norepinephrine infusion
% = Per cent change in each patient expressed as per cent of control value (Control = 100%)
Nor. = Norepinephrine

TABLE 1B.—EFFECT OF NOREPINEPHRINE ON MEAN BLOOD PRESSURE, RENAL BLOOD FLOW AND RENAL VASCULAR RESISTANCE

	RBF			*MBP*			*RVR*			
Patient	*C*	*NE*	*%*	*C*	*NE*	*%*	*C*	*NE*	*%*	*Dose γ/KG./MIN.*
Nor. 1	1698	891	52	94	141	150	.055	.158	287	.188
Nor. 2	1025	732	71	96	126	131	.094	.172	183	.252
Nor. 3	1050	668	64	92	128	139	.088	.192	218	.483
Nor. 4	1483	850	57	109	143	131	.073	.168	230	.156
Nor. 5	617	503	82	100	131	131	.162	.260	160	.380
Nor. 6	1221	744	61	100	138	138	.082	.185	226	.346
Nor. 7	1064	600	56	105	133	127	.099	.222	224	.222
Nor. 8	1093	888	81	98	133	136	.090	.150	167	.360
Nor. 9	1039	467	45	80	115	144	.077	.246	319	.501
Mean	1143	705	63	97	132	136	.091	.195	224	.321

See Table 1A for key to abbreviations.

RBF = Renal Blood Flow: $\frac{\text{RPF}}{\text{1-hematocrit}}$

MBP = Mean Blood Pressure: one-third pulse pressure plus diastolic pressure

RVR = Renal Vascular Resistance: $\frac{\text{MBP}}{\text{RBF}}$

TABLE 2.—EFFECT OF NOREPINEPHRINE ON GLOMERULAR FILTRATION RATE AND MAXIMUM TUBULAR TRANSPORT OF P-AMINOHIPPURATE*

	UV			*GFRin*			*GFRcr*			*TmPAH*			*GFR±/TmPAH*			*MBP*			
	C	*NE*	%	*C*	*NE*	%	*C*	*NE*	%	*C*	*NE*	%	*C*	*NE*	%	*C*	*NE*	%	*Dose γ/KG./MIN.*
Nor. 10	6.7	7.3	109	79**	85	108	80	73	91	73	62	85	1.08	1.37	127	108	156	144	.254
Nor. 11	10.5	7.6	72	74	79	107	78	77	99	89	74	83	0.83	1.07	129	88	114	130	.773
Nor. 12	9.2	3.5	38	118	87	74	108	86	80	45	50	111	2.62	1.74	66	91	124	136	.282
Nor. 13	5.5	4.0	73	105	84	80	103	92	89	45	46	102	2.33	1.83	79	95	114	120	.450
Nor. 14	4.2	9.3	221	108	89	82	122	84	69	70	67	96	1.54	1.33	86	107	133	124	.440
Nor. 15	3.4	3.3	97	115	91	79	97	82	85	51	54	106	2.25	1.69	75	106	137	129	.654
Nor. 16	11.8	14.1	119	80	73	91	85	86	101	47	50	106	1.70	1.46	86	83	134	161	.444
Nor. 17	8.0	11.0	138	87	86	99	83	80	96	102	84	82	0.85	1.02	120	112	138	123	.443
Nor. 18	3.6	10.8	300	117	124	106	125	115	92	101	107	106	1.16	1.16	100	85	134	158	.314
Nor. 19	6.8	9.8	144	127	115	91	128	113	88	78	75	96	1.63	1.53	94	113	145	128	.397
Nor. 20	7.5	4.2	56	106	91	86	105	93	89	70	77	110	1.51	1.18	78	86	136	158	.267
Nor. 21	3.2	3.8	119	111	112	101	103	90	87	58	56	97	1.91	2.00	105	84	125	149	.407
Nor. 22	1.8	2.0	111	24	24	100	21	22	105	13	12	92	1.85	2.00	108	84	105	125	.377
Nor. 23	8.9	8.6	97	27	25	93	25	19	76	13	17	131	2.08	1.47	71	111	136	123	.400
Nor. 24	2.7	2.6	.96	23	17	74	25	20	80	10	14	140	2.30	1.21	53	100	140	140	.664
Mean	6.3	6.8	119	87	79	91	86	75	88	58	56	103	1.71	1.47	92	97	131	137	.438

* For Key to Abbreviations see Tables 1A and 1B.
** Mannitol Clearance: Glomerular filtration rate: cc./min.
GFRin = Inulin Clearance: Glomerular filtration rate: cc./min.
TmPAH = Maximal tubular function: mg. PAH secreted by the tubules per minute
± GFR = Inulin Clearance

TABLE 3.—EFFECT OF EPINEPHRINE ON GLOMERULAR FILTRATION RATE AND MAXIMUM TUBULAR TRANSPORT OF P-AMINOHIPPURATE*

	UV			*GFRin*			*GFRcr*			*TmPAH*			*GFR±/TmPAH*			*MBP*			
	C	*E*	%	*C*	*E*	%	*C*	*E*	%	*C*	*E*	%	*C*	*E*	%	*C*	*E*	%	*Dose γ/KG./MIN.*
Epi. 1	3.0	4.7	157	101	116	115	114	125	110	85	84	99	1.19	1.38	116	78	109	140	.253
Epi. 2	5.4	8.2	152	141	145	103	...	...	...	88	90	102	1.60	1.61	101	87	117	134	.282
Epi. 3	8.2	10.8	132	140	127	91	114	113	99	72	72	100	1.94	1.76	91	106	109	103	.304
Epi. 4	3.0	4.4	147	96	101	105	116	125	108	81	78	96	1.19	1.29	108	91	95	104	.213
Epi. 5	3.1	3.7	119	93	97	104	82	77	94	76	75	99	1.22	1.29	106	88	97	110	.139
Epi. 6	4.9	10.1	206	100	90	90	100	91	91	47	48	102	2.13	1.88	88	116	113	97	.265
Mean	4.6	7.0	152	112	113	101	105	106	100	75	75	100	1.55	1.54	102	94	107	115	.243

* For Key to Abbreviations see Tables 1A, 1B, and 2.
Epi. = Epinephrine
E. = Average values during epinephrine infusion
± = GFR-Inulin Clearance

The effect of norepinephrine on GFR and maximum tubular transport of p-aminohippurate (TmPAH) was determined in 15 patients (Table 2). These patients received a larger amount of the drug (0.438 micrograms/kg./min.), but had a comparable rise in mean blood pressure. Although the average value for GFR after norepinephrine was only slightly lower than the control value, in 5 of the patients there was a significant decrease in GFR (15% or more). With the exception of 3 patients (Nor 10, 11, and 17), there was no significant reduction in TmPAH, and in these 3 patients there was no change in glomerular filtration rate.

Six patients received epinephrine intravenously in an average dose of 0.243 micrograms/kg./min. (Table 3). Although there was only a 15% increase in the average mean blood pressure, all but one of the patients had an elevation of the systolic pressure greater than 30%. The failure of the calculated mean blood pressure to increase in a parallel degree to the systolic pressure was due to the fact that the diastolic pressure was usually unchanged or even decreased (Fig. 1). In this group there was no change in either GFR or TmPAH, but urine flow was increased on the average of 152% of the control value.

EFFECT OF NOREPINEPHRINE AND EPINEPHRINE ON BLOOD PRESSURE

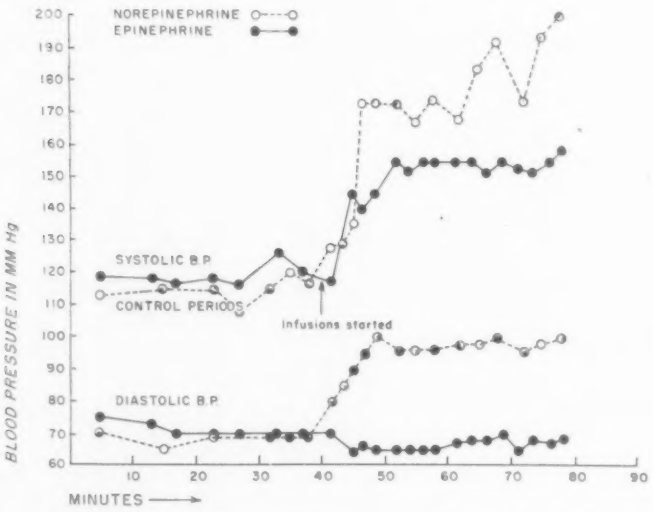

Fig. 1.—Typical blood pressure response to intravenous infusion of norepinephrine and epinephrine. Data from same patient on different occasions. Note failure of diastolic blood pressure to increase after epinephrine in contrast to the sharp increase after norepinephrine.

In order to determine if the above observations on renal hemodynamic effects of norepinephrine were similar in patients who had previously been made hypotensive, 8 hypertensive and 7 normotensive subjects were given hexamethonium chloride or bromide. This produced an average decrease in mean blood pressure of 31% (range 14% to 52%) (Table 4), and in 2 patients there were definite symptoms of

cerebral ischemia. After the blood pressure had stabilized, and renal clearances had been measured, an infusion of norepinephrine was begun, the rate being gradually increased until the blood pressure approached control levels. In most of the patients the necessary dosage was much less than that required to produce a similar rise in normal subjects. No studies were made, however, to determine if this was a result of sensitization by hexamethonium or if rather it was associated with the pre-existing hypertensive state. Goldenberg *et al.* have previously observed that hypertensive patents were much more sensitive to norepinephrine than normotensive individuals[4]. The pattern of renal response was variable, but usually GFR was increased to or toward control levels if it had been significantly depressed during the hexamethonium periods, or unchanged, if it had remained near control levels during administration of hexamethonium. In 2 patients (Nor 29 and 38) there was a decrease in GFR during hexamethonium administration with a further decrease during the administration of norepinephrine. Renal plasma flow was usually reduced somewhat and renal vascular resistance was increased, similar to the normal control subjects. As in the other groups TmPAH was not consistently altered by either drug.

Discussion. The response in these subjects indicated that norepinephrine produced significant renal vasoconstriction. Although specific segmental resistances were not measured, the failure of glomerular filtration rate to increase with rising systemic blood pressure is evidence of afferent arteriolar constriction, and likewise the consistent elevation of the filtration fraction in this circumstance is indicative of efferent arteriolar constriction. This type of response, characterized by a maintained or slightly reduced GFR and a marked depression in RPF has been observed after the administration of epinephrine[1,7], Paredrinol[7], ephedrine[7], and phenylephrine[2] to human subjects. In contrast, however, administration of norepinephrine or epinephrine to dogs[5] has produced a very marked reduction in both GFR and RPF. The latter studies were done in essentially the same way as the current ones on humans.

The lack of effect of norepinephrine and epinephrine on functioning tubular mass (TmPAH) in this study is in agreement with the results of Smith[9] who found no change in TmG* or TmD* after the administration of epinephrine to human subjects. It is in contrast to the results obtained with a continuous infusion of norepinephrine and epinephrine to dogs[5] in which after a slight initial depression of GFR and the GFR/TmG ratio, there was a proportional and progressive decrease in both GFR and TmG, with a maintained GFR/TmG ratio. Whereas the results in human studies indicate a reduction in blood flow through each individual nephron without altering the number of active nephrons (reduction in blood flow, without a change in tubular mass: Fig. 2B) the dog studies indicate a reduction in the number of active nephrons as reflected in the reduction in functioning tubular mass (Fig. 2A). The possibility exists that similar changes might have occurred in the human subjects with larger doses of the drugs. However, the fact that they did not occur in any of the subjects, although some had comparable rises in blood pressure (to the dog studies) militates strongly against this possibility, and would indicate that there is a fundamental difference be-

* P-aminohippurate used in the current study to measure maximum tubular function rather than glucose or diodrast.

TABLE 4.—EFFECTS OF NOREPINEPHRINE ON RENAL HEMODYNAMICS AFTER GANGLIONIC BLOCKADE*

	UV			*GFRin*			*RPF*			*FFin*			*RBF*			*MBP*			*RVR*		
	C	C_6	*NE*	*C*	C_6	*NE*	*C*	C_6	*NE*	*C*	C_6	*NE*	*C*	C_6	*NE*	*C*	C_6	*NE*	*C*	C_6	*NE*
Nor. 25#	0.9	1.0	0.6	70	11	47	260	37	133	.269	.297	.353	487	66	257	165	95	169	.339	1.439	.658
Nor. 26#	10.2	0.6	10.5	67	44	75	390	445	400	.172	.099	.188	780	840	800	131	63	146	.168	.075	.183
Nor. 27	1.4	0.6	2.5	167	112	154	644	726	655	.259	.154	.235	1109	1252	1091	88	68	125	.079	.054	.115
Nor. 28	4.2	0.4	0.4	111	75	98	895	596	616	.124	.126	.159	1627	1084	1081	105	56	92	.065	.052	.085
Nor. 29#	1.0	0.9	0.5	23	18	10	111	96	34	.207	.188	.294	163	140	49	143	99	153	.877	.707	3.122
Nor. 30	1.3	2.8	1.1	117	94	108	592	497	412	.198	.189	.262	1096	873	750	77	66	113	.070	.076	.151
Nor. 31#	...	...	...	125	110	115	477	623	515	.262	.177	.223	829	952	787	139	107	161	.168	.112	.205
Nor. 32#	...	...	...	62	57	53	162	272	252	.383	.210	.210	291	444	430	170	123	174	.584	.277	.405
Nor. 33	3.1	1.2	1.0	109	101	98	620	668	561	.176	.151	.175	1240	1309	1144	83	57	112	.067	.044	.098
Nor. 34	1.0	0.4	0.6	92	88	86	400	355	339	.230	.248	.254	755	634	606	104	81	129	.138	.128	.213
Nor. 35#	0.6	0.9	4.0	35	34	44	354	447	413	.099	.076	.107	528	661	607	130	100	184	.246	.151	.303
Nor. 36	5.1	0.7	2.3	87	95	126	747	696	829	.116	.136	.152	1265	1141	1427	86	56	115	.068	.049	.081

				TmPAH			*GFR/TmPAH*													
Nor. 37	5.3	2.3	2.5	27	18	17	20	27	24	1.35	0.67	0.71	..	..	..	107	69	119	..	..	..
Nor. 38#	...	...	...	78	59	45	46	38	40	1.70	1.55	1.13	..	..	..	180	137	176	..	..	..
Nor. 39#	7.5	3.1	3.9	120	104	108	81	79	78	1.48	1.32	1.38	..	..	..	123	76	128	..	..	..

* For Key to Abbreviations see Tables 1A, 1B, and 2.
C_6 = Average values during ganglionic blockade by hexamethonium.
= Patients with hypertension.

tween the responsiveness of the human and the canine kidney.*

There was no evidence in the present study to suggest that the reduction in renal plasma flow was the result of a renal vascular shunt of the nature suggested by Trueta[10] since if any significant number of renal tubules had been by-passed, there would have been a proportional reduction in TmPAH. Although one might object to the validity of this argument on the basis that RPF and TmPAH were not measured simultaneously in any of the patients, there is no reason to suspect that renal plasma flow was not reduced in those in whom TmPAH was measured, as judged by the consistent and marked reduction in plasma flow in those in whom it was determined.

The marked reduction in RPF and the occasional reduction in GFR observed in normotensive subjects after norepinephrine, might at first appear to contraindicate the use of this drug in the treatment of patients with severe hypotension who have depressed renal function on the basis of inadequate fil-

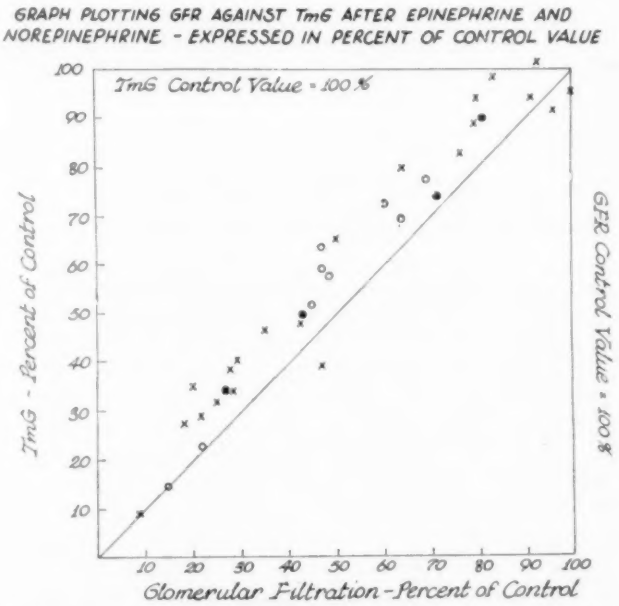

Fig. 2A.—Observations on dogs during continuous infusion of norepinephrine and epinephrine. Maximum tubular function (TmG) expressed as percent of control is plotted against glomerular filtration rate (GFR) during the continuous infusion of norepinephrine and epinephrine in dogs. The rate of infusion was increased every 20 minutes in an incremental fashion so that 2 collection periods were obtained between each change in the rate of infusion. The symbols are as follows: a. O = epinephrine, and ● = norepinephrine during successive periods showing the best agreement. b. X = both epinephrine and norepinephrine during periods of infusion in which successive periods did not show a similar degree of stability as in (a). There is a progressive reduction in both GFR and TmG as the rate of infusion is increased and the blood pressure progressively elevated. (Courtesy of Circulation).

* The fact that p-aminohippurate was used to measure maximum tubular function in the current study rather than glucose as was used in the animal work may account for minor differences.

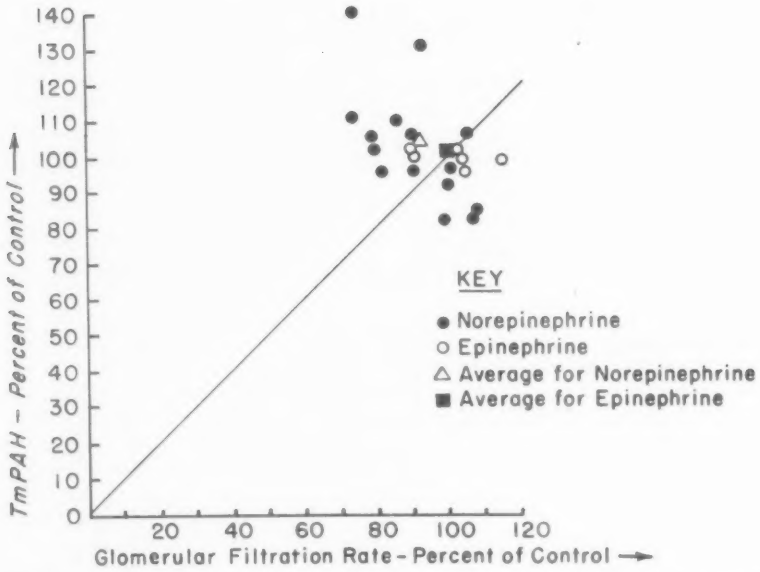

Fig. 2B.—Observations on human subjects during the continuous infusion of norepinephrine and epinephrine. Maximum tubular function (TmPAH) (expressed as percent of control) is plotted against glomerular filtration rate expressed in the same manner. (Tables 2 and 3). Although there appears to be some instability, this appears to be a random response.

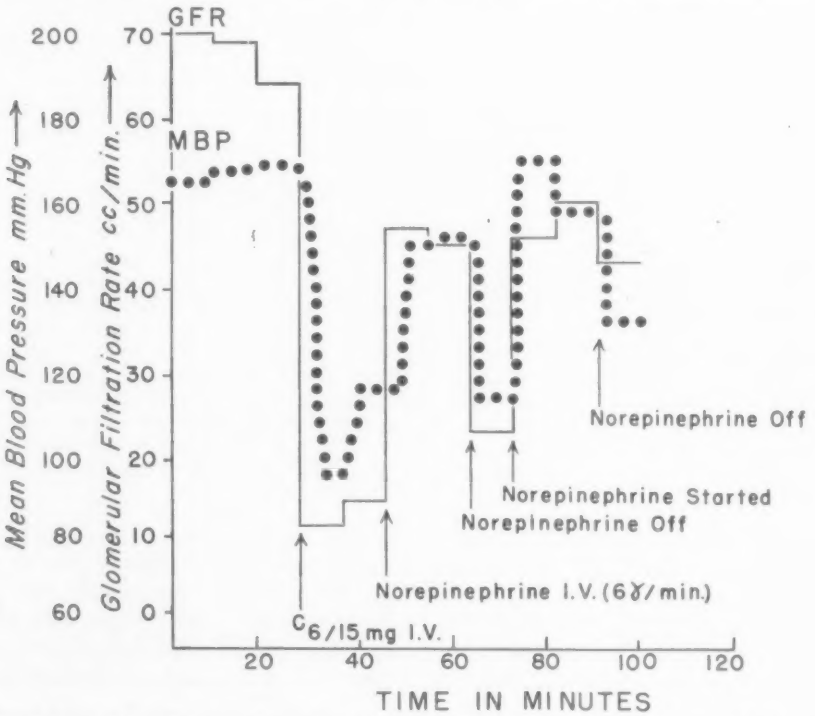

Fig. 3.—Patient (Nor 25) in whom the glomerular filtration rate was depressed following a marked reduction in blood pressure with hexamethonium. Whenever the blood pressure was elevated by the administration of norepinephrine the filtration rate increased. (Courtesy of the Journal of Clinical Investigation).

tration pressure. However, clinical observations in such patients[8], and the results obtained in patients with hypotension due to ganglionic blockade (albeit not the same as surgical or other types of shock) indicate that the net effect may be quite different in the presence of hypotension (Table 4). When the glomerular filtration rate was markedly reduced by hypotension due to hexamethonium, blood pressure elevation with norepinephrine actually resulted in an increased rate of filtration. Also, in anuric or oliguric patients in whom blood pressure has been restored to normotensive levels with norepinephrine[8], it has not been uncommon to observe a return of urine flow toward normal. The very fact that an anuric patient begins to have a significant urine output is evidence of an improvement in renal function. These observations suggest that in patients in whom there is severe hypotension, following elevation of the systemic blood pressure, the net result may be an improvement in renal function.

Conclusions. 1. Administration of norepinephrine to normotensive human subjects produced a depression of renal plasma flow, but had little effect on glomerular filtration rate in the majority of patients. Renal vascular resistance and the filtration fraction were significantly elevated. There was no significant change in maximum tubular transport of p-aminohippurate after either norepinephrine or epinephrine.

2. These changes were thought to be a result of an increase in both afferent and efferent arteriolar resistance, so that there was reduced blood flow through each individual nephron. There was no evidence to suggest either a decrease in the number of active nephrons as had been previously observed in dogs or a renal vascular shunt by-passing cortical nephrons as described by Trueta. These results indicate that there is probably a fundamental difference in responsiveness between the dog and the human kidney.

3. The response of patients to norepinephrine during the hypotensive state produced by ganglionic blockade was variable. Generally in the patients who had marked depression of renal function, there was a return of glomerular filtration rate toward control values. This suggests that in the treatment of severe hypotensive states seen clinically, elevation of blood pressure to normotensive levels, may overbalance the renal vasoconstrictor effect of norepinephrine, to a degree that renal function will be improved.

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DIFFERENTIATION BETWEEN CONGENITAL AND ACQUIRED FORMS OF HEMOLYTIC ANEMIA*

Observations on 47 Cases of Hereditary Spherocytosis and 24 Cases of Autoimmune Hemolytic Disease

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Use of newly developed laboratory tests[22] has resulted in detection of many cases of hemolytic anemia which until recently would have been improperly or inadequately classified. Accelerated destruction or shortened life span of red blood cells is now recognized as a much more common cause of anemia than was formerly appreciated[14,16,34,39,42,59].

The purpose of this paper is to characterize and differentiate a specific type of congenital hemolytic anemia (hereditary spherocytosis) and a specific type of acquired hemolytic anemia (autoimmune hemolytic disease). The observations summarized are based on experience with 47 cases of the former and 24 cases of the latter type of hemolytic disease encountered in this clinic since 1944. These disorders are representative of the two principal categories of hemolytic disease, one supposedly due to an intrinsic abnormality of the red cell, the other to the action of abnormal globulins and perhaps other extrinsic factors. It will be emphasized that, although there are striking differences between these two disorders with respect to both clinical and laboratory findings, differential diagnosis may be difficult at times in any given case. Moreover, atypical forms of hemolytic disease, either congenital or acquired, may be encountered which do not fulfill the criteria for diagnosis of either of the two specific conditions to be discussed or of other well defined hemolytic disorders.

Red blood cells may have shortened life span because of either intracorpuscular abnormalities or the action of extracorpuscular factors. Intracorpuscular abnormalities are encountered in hereditary spherocytosis (congenital hemolytic anemia)[9], sickle cell anemia[2,33,45], Mediterranean anemia[24], nocturnal hemoglobinuria[22], and in several unnamed chronic hemolytic disorders, both hereditary and nonhereditary[5,46]. The defective red cells from these patients are rapidly destroyed when transfused into normal recipients, while normal red cells usu-

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ally (but not always) survive normally after transfusion into such patients. On the other hand, normal red corpuscles may be rapidly eliminated after transfusion into patients whose own red cells are being destroyed by the action of extrinsic factors such as antibodies[27, 42,43,55].

Hereditary Spherocytosis. The disorder known as congenital hemolytic anemia is more appropriately called hereditary spherocytosis (HS) since it is inherited as a Mendelian dominant and is characterized by the presence of spherocytes. The abnormal thickness of the spherocyte may actually be a manifestation of a metabolic abnormality of the cell, but no specific anomaly of cellular metabolism has been demonstrated to date.

FRAGILITY OF ERYTHROCYTES. The abnormal osmotic fragility (O.F.) and mechanical fragility (M.F.) of the red cells in hereditary spherocytosis are well known[22]. Representative O.F. curves are shown in Fig. 1 in which per cent hemolysis is plotted against concentration of sodium chloride. If the blood being tested contains many circulating spherocytes, a large portion of the O.F. curve is shifted to the left of the normal range in such a graph. A small portion of the curve, often called a "tail", is shifted to the left in less active cases having fewer spheroidal cells in the circulation. In 13 of our 47 cases the O.F. curve for freshly drawn red cells was in or near the normal range. An abnormally great shift to the left (Fig. 2) was observed in these cases, however, when the cells were tested after sterile incubation at body temperature for 24 hours. The O.F. test with incubated red cells thus brings to light an otherwise questionable or inapparent abnormality of the red corpuscles in some cases[22,53].

Mechanical fragility of the red cells is determined according to the method of Shen, Castle and Fleming[41] by measuring the amount of hemolysis in a small sample of blood placed in a rotating Erlenmeyer flask containing glass beads. Mechanical fragility of the red cells from patients with hereditary spherocytosis is nearly always greater than normal and may reflect increased susceptibility to destruction of such cells by the wear and tear of circulation[53]. Increase in M.F. after incubation of the spheroidal cells is usually, but not always, marked.

LYSIS OF INCUBATED RED CELLS. When sterile defibrinated blood from patients with HS is placed in an incubator at body temperature for 48 hours the red cells undergo lysis to a much greater extent than in normal blood similarly treated[7,8,53,54]. Although the mechanisms by which hemoglobin is released from red cells during such incubation are not entirely clear, it can be stated that lysis is not due to complement activity or to swelling of the cells. During the second 24-hour period of incubation the cells actually shrink to approximately their original volume. Abnormal results have been obtained much more consistently in HS than in other hemolytic disorders encountered in our clinic but the lack of specificity of this simple test should be emphasized.

ROLE OF THE SPLEEN AND MODIFYING FACTORS. Studies employing both transfusion and perfusion have revealed that the abnormally thick red cells from patients with HS are selectively trapped in the spleen[17,60], possibly because of their inability to escape through slit-like openings from the pulp to the venous sinusoids[49]. While stagnating within the spleen, the spheroidal cells may undergo changes like those observed in blood incubated *in vitro*[23]. Many of the red cells probably disintegrate within the pulp and some may be destroyed by phagocytosis. Others manage to escape, but after long confinement they may be suscep-

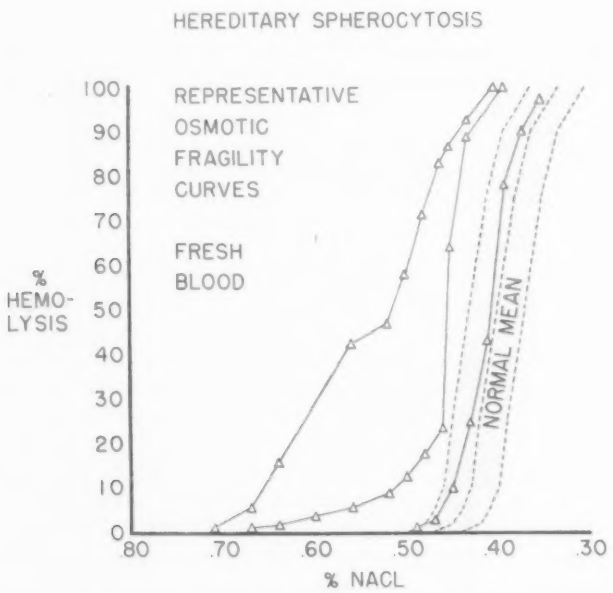

Fig. 1.—Representative O.F. curves obtained with fresh blood from patients with hereditary spherocytosis. The curve to the left reveals increased fragility of a large portion of the patient's red cells; the middle curve is "tailed", indicating increased fragility of a small portion of the red cell population. The curve to the right is in the upper normal range (mean $\pm 2\sigma$ shown in shaded area).

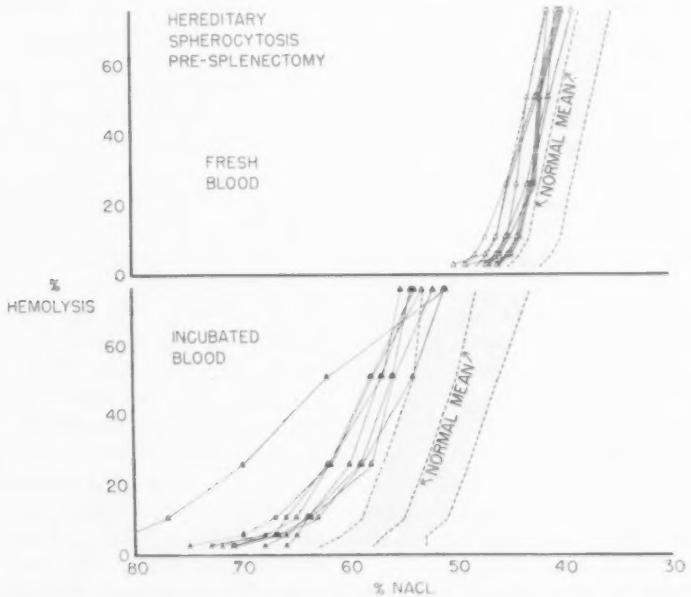

Fig. 2.—O.F. curves obtained with freshly drawn and incubated blood from 8 selected patients with hereditary spherocytosis. All of the curves for fresh blood are in or near the normal range, while all of the curves for incubated blood are clearly outside the normal range (mean $\pm 2\sigma$ shown in shaded area).

tible to destruction by wear and tear of active circulation.

The extent to which spherocytes are destroyed by phagocytosis in the spleen is difficult to quantitate[15]. It should also be emphasized that the mechanisms by which crisis occurs in HS are not clear[1]. Further studies are especially needed to explain the sudden occurrence of aplasia[32] or arrested maturation[11] of erythroid cells in the marrow in some patients during crises. The shortened survival time of transfused normal red cells demonstrated in a few instances also requires additional investigation[11,50]. "Warm autoantibodies" in the serum or attached to the red cells have been reported in a small number of cases, but are usually lacking[31,44,51]. Tests for such antibodies have been positive in only one of 47 cases investigated in the Strong Memorial Hospital. The affected relatives of our single exceptional case had hematologic findings typical of hereditary spherocytosis, but antiglobulin tests with their red cells were negative.

Among persons afflicted with HS the severity of the hemolytic process varies widely, perhaps due to differences in gene penetrance or other modifying factors[53]. Some persons are so mildly affected that the presence of abnormal red cells is overlooked unless tests are carried out with incubated red cells. Among relatives of severely anemic patients we have encountered cases lacking splenomegaly, anemia, jaundice, reticulocytosis, and showing very little spherocytosis or increase in O.F. in fresh blood samples. Diagnosis in such cases has been based on (a) slightly increased M.F. of fresh blood, (b) increased O.F. of incubated blood and (c) marked lysis of blood placed in an incubator for 48 hours.

Anemia and jaundice are completely and permanently relieved after splenectomy in practically all cases regardless of the rapidity of red cell destruction prior to operation. This was true in each of the 35 splenectomized patients in our series. Spherocytosis persists indefinitely but is usually less marked and is not associated with excessive hemolysis once the splenic trap is removed. Abnormality of the red corpuscles after splenectomy is best demonstrated by measuring the O.F. of incubated blood samples. The mean O.F. of incubated red cells in our experience, is actually greater after splenectomy than before (Fig. 3)[53].

Other laboratory and clinical features of this disorder can best be cited in making comparisons with the findings and course of the acquired hemolytic state.

Autoimmune Hemolytic Disease. DEFINITION. Since the meaning of the term acquired hemolytic anemia varies from one author to another, use of a more accurately descriptive term is advisable. The type of acquired disorder under consideration in this paper might better be called autoimmune hemolytic disease (AHD) because it is characterized by the presence of circulating globulins capable of reacting with the patient's own red cells[56]. Although cases in which the autoantibodies are active only at low temperatures are included in this category by some authors, we shall confine our attention in this paper to cases in which the autoantibodies showed maximal activity at body temperature. Whether or not these globulins are really antibodies is debatable, and if they are antibodies, the nature of the antigenic stimulus provoking their formation is not clear. It has nevertheless been customary to refer to them as autoantibodies since they react with the patient's own red cells and this custom will be followed in this paper for the sake of simplicity. As in the hereditary malady just described, the words "anemia" and "jaundice" are best deleted from the name of the disorder

since either anemia or jaundice or both may be lacking at times in a large share of the patients studied.

The abnormal globulins may develop independently or may be found in association with disorders of collagenous[12] or reticuloendothelial tissues[38]. Since hemolytic anemia associated with demonstrable autoantibodies may be the first manifestation of some of these disorders, search for an underlying disease such as periarteritis or lymphoma should be unrelenting. For example, one of the patients in our clinic was recently found to have reticulum cell sarcoma after having been followed closely for 8 years because of periodic hemolytic anemia. The occurrence of autoantibodies in patients who have, or ultimately develop, such tumors has provoked considerable speculation.

DEMONSTRATION OF AUTOANTIBODIES AND OTHER ABNORMAL SERUM GLOBULINS. Demonstration of autoantibodies in the patients serum or attached to the erythrocytes is possible by employing methods developed largely since 1945. It should be emphasized however, that either false-positive or false-negative results may be obtained if the importance of certain technical details is not fully appreciated.

The antiglobulin test (Moreschi[30]

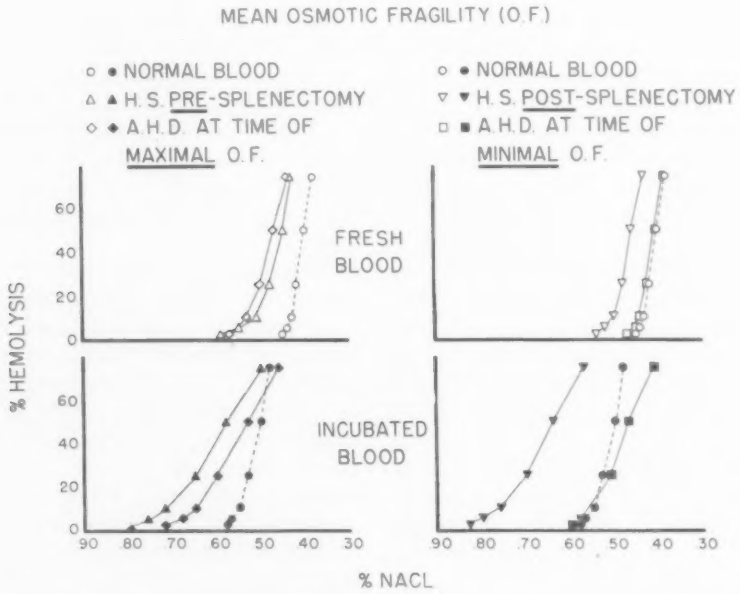

Fig. 3.—Plot of mean concentrations of sodium chloride causing 2, 5, 10, 25, 50 and 75% hemolysis of freshly drawn and incubated red cells from 26 normal donors; 17 patients with HS pre-splenectomy; 12 patients with HS post-splenectomy; 13 patients with AHD at time of maximal O.F. and 12 patients with AHD at time of minimal O.F. Note that the increase in O.F. after incubation of the red cells is much greater in HS than in AHD or in normal blood and that in HS the increase in O.F. with incubation is even greater after splenectomy than before. Mean figures for the AHD group were computed and plotted without respect to the presence or absence of the spleen at the time of the O.F. determinations. The fact that 6 of the 12 AHD cases tested at the time of minimal O.F. had been splenectomized presumably accounted for the less than normal increase in O.F. of the incubated red cells in this group.

and Coombs, Mourant, and Race[3]) is the most sensitive method known for detection of certain erythrocyte-bound globulins. The patient's red cells coated with globulin (presumably antibody globulin) are agglutinated by antiglobulin serum prepared from rabbits immunized with human gamma globulin. The prozone phenomenon may be observed at times[47] and there are variations in reactivity of different lots of rabbit serum[28]. Critical testing therefore requires the use of several different antiglobulin sera, each of which has been optimally diluted[36].

Globulin attached to the patient's red cells can also be demonstrated by elution from the cells or from the erythrocyte stroma followed by appropriate tests of the eluate with normal red cells[10,25,26,28]. Such eluates have been prepared from the red cells or stroma in 17 of 19 cases in our group in which it was attempted one or more times. In our experience detectable amounts of such globulin could not always be eluted from red cells agglutinable by antiglobulin serum and could never be eluted from red cells giving negative antiglobulin reactions. The eluted globulin was demonstrable by sensitization of normal red cells for the antiglobulin tests or by agglutination of trypsin-treated normal red cells.

Oxalated blood from patients with AHD frequently has a granular appearance when examined grossly or with a hand lens as the blood is made to flow in a thin layer by tilting the bottle. Autoagglutination in such cases is attributable to coating of the red cells with antibody globulin but the lack of specificity of this crude test should be emphasized. Equally marked macroscopic aggregation of red cells is sometimes produced by rouleaux-forming substances in diseases such as multiple myeloma, periarteritis nodosa and acute rheumatic fever.

Antibodies in the patients' sera are frequently capable of agglutinating trypsin-treated normal human red cells[10,37,51,56]. Lysis of trypsin-treated erythrocytes in the presence of human complement is produced by sera of some patients but this has been the exception rather than the rule in our experience. When the pH of the patient's serum is reduced *in vitro* to about 6.8, the serum will sometimes directly agglutinate normal red cells and will more frequently sensitize normal red cells for the antiglobulin reaction[10,20,22,28,56]. During episodes of hemolysis the titer of autoantibody in the serum frequently increases and may remain increased for some time after deceleration of red cell destruction.

Cold agglutinins, cold hemolysins, sheep cell agglutinins and serum components capable of giving false-positive serological tests for syphilis may be encountered in addition to the "warm" autoantibodies with which we are concerned in this paper[10,28]. Isoantibodies, such as anti-Rh, may develop readily after multiple transfusions and may complicate cross-matching and transfusion therapy in these individuals[29]. An abnormality in production of plasma globulins is thus an outstanding feature of this disorder. In some cases cold precipitable serum protein may also be produced but these so-called "cryoglobulins" have not been implicated in destruction of red cells[4,28].

Complement may be markedly deficient at times in the serum of patients with AHD, presumably because it is fixed to the red cells with antibody globulin, but the possibility of impaired formation of complement has not been excluded. Serum complement has been measured on one or more occasions in 17 of our cases. In 2 of the patients complement activity was nearly absent during even relatively quiescent phases of the disease and in

a third patient there was significant decrease in complement activity during the initial hemolytic episode.

VARIATIONS IN HEMATOLOGIC FINDINGS. During episodes of hemolysis spherocytes usually appear and O.F. and M.F. may be increased but are less regularly increased than in hereditary spherocytosis[56,57]. Increases in O.F. and M.F. with incubation of the red cells are much less marked than in the hereditary disorder and during quiescent periods O.F. and M.F. of both freshly drawn and incubated red cells are normal (Fig. 3).

Hemoglobinemia and methemalbuminemia may be demonstrable during crises in the autoimmune disease and presumably are the result of intravascular hemolysis[6,57]. These findings are especially prominent after transfusion since the relatively mature donated red cells may be more susceptible to the action of the antibody than are the patient's own relatively immature erythrocytes[20,57].

VARIABLE COURSE AND RESPONSE TO SPLENECTOMY AND HORMONE THERAPY. In 8 of the adult cases of AHD observed in our clinic, hemolytic episodes have recurred at irregular intervals, sometimes weeks apart, sometimes years apart[58]. The red cells have been consistently agglutinable by antiglobulin serum over periods of years in the majority of the chronic cases, even during periods when the rate of red cell turnover appeared to be normal or nearly normal[56,57].

The importance of the spleen as a source of antibody and/or as a site of red cell destruction may vary widely from patient to patient, thus explaining the unpredictable variation in response to splenectomy[10,15,28,45,48,54]. Splenectomy has been performed in 11 of our 24 cases of AHD since 1944. There has thus far been no recurrence of hemolytic anemia in 6 of the patients, 3 of whom have been observed for only one year, one for 3 years, one for 4 years, and one for 9 years since operation. In 4 of the 11 patients, however, hemolysis recurred one or more times after operation, the intervals ranging from a few weeks to 2 years. One patient died during a hemolytic crisis 36 hours after an emergency splenectomy was performed under unfavorable conditions.

Deceleration of red cell destruction in many patients with AHD has followed administration of ACTH and cortisone in numerous clinics since 1950[4,13,21,40,57]. Although the antiglobulin tests of the patients' red cells have sometimes become negative and the titers of the serum autoantibody have frequently diminished after hormone therapy, the exact mechanisms by which ACTH and cortisone act in AHD are not clear. Evaluation of any form of treatment of AHD is difficult because of the naturally occurring fluctuations in hemolytic activity and because many of the patients ultimately succumb to an underlying disease such as leukemia.

Eighteen of our patients with AHD have been treated with ACTH or cortisone since March 1950. Remission of the hemolytic process followed administration of ACTH or cortisone in 13 cases, in 9 of which remissions were well sustained without maintenance therapy. In 5 of the 18 cases in which hormone treatment was given, the results were rather unsatisfactory or difficult to evaluate because of complications. Our experience as a whole supports the current practice of giving ACTH or cortisone in most active cases, either symptomatic or idiopathic.

Comparison of Hereditary Spherocytosis and Autoimmune Hemolytic Disease. The principal features of hereditary spherocytosis (HS) and autoimmune hemolytic disease (AHD) are summarized in Table 1. The general features listed require no further

TABLE 1.—PRINCIPAL FEATURES OF HEREDITARY SPHEROCYTOSIS (HS) AND AUTOIMMUNE HEMOLYTIC DISEASE (AHD)

General	*HS*[53,54]	*AHD*[18,40,54,56,57]
Inheritance	Mendelian dominant[35]	None demonstrable
Sex incidence	Equal	More common in females
Associated disorder	Usually none	Frequent complication of disorders of collagenous and reticuloendothelial tissues
Splenomegaly	Usually moderate	Usually moderate
Skeletal abnormalities	Rare	None
Leg ulcers	Occasionally seen	Rare
Thrombophlebitis	Uncommon	Common[57,58]
Cholelithiasis	Common	Common
Response to splenectomy	Complete and permanent relief of anemia and jaundice	Irregular; frequent recurrence of hemolysis
Response to ACTH or cortisone	No reports	Frequently good, unless patient succumbs to associated disease
Hematologic Findings		
Anemia	Variable; may be lacking	Variable; may be lacking
Spherocytosis	? Regularly present; slight in some cases	Often marked during crisis; absent during quiescence
Osmotic fragility	Usually increased with fresh cells; regularly increased with incubated cells even during quiescence and after splenectomy	Irregularly increased with both fresh and incubated cells during crises; normal during quiescence
Mechanical fragility	Regularly increased	Usually increased during crises; normal during quiescence
Lysis of incubated red cells	Marked at 48 hours in all cases before and after splenectomy	Marked only in occasional case during severe crisis
Rate of destruction of normal transfused red cells in patient	Usually, but not always, normal	Usually rapid in active cases
Trapping of red cells in spleen	Selective for patient's cells	Both patient's and donated cells
Hemoglobinemia and methemalbuminemia	? None	Common during active phases, especially after transfusion
Nucleated red cells in circulation	In active cases before splenectomy. None after splenectomy	In active cases. Much more numerous after splenectomy if hemolysis persists or recurs
Thrombocytopenia and leukopenia	Occasionally present; usually mild	Occasionally present; may be severe[19]
Serologic Findings		
Antiglobulin test with patient's red cells	Usually negative	Usually positive, with frequent persistence during quiescence
Antibody elutable from patient's red cell stroma	No reports	Frequent
Autoagglutination	Usually absent	Frequently marked especially during hemolytic episodes
"Warm" autoantibody in patient's serum	Usually absent	Frequently present especially during and after hemolytic episodes
Other abnormal antibodies (auto-, iso-, and hetero-) in patient's serum	Rare	Frequent
Cold precipitable protein in serum	None	Occasionally present in patients with myeloma, lymphoma and lymphocytic leukemia
Complement in patient's serum	Normal	Sometimes markedly deficient
Phagocytosis of red cells by monocytes and mature granulocytes in incubated venous blood	None observed[57]	Frequently marked in active cases[57]

comment. Abnormality of the red cells persists after splenectomy in HS despite the uniformly good clinical response. In AHD, on the other hand, hematologic abnormalities are observed during periods of accelerated red cell destruction but disappear during quiescent phases of the disease. Fluctuations in the rate of red cell destruction frequently continue after splenectomy in patients with AHD.

Abnormal autoantibodies are found only in exceptional cases of HS while in AHD demonstration of autoantibodies in the serum or attached to the red cells is the prime requisite for diagnosis.

Conclusion. Differentiation between hereditary spherocytosis (a congenital hemolytic anemia) and autoimmune hemolytic disease (an acquired hemolytic anemia) can usually be made with accuracy because there are striking clinical, hematologic and serologic differences. The differences become especially apparent when the patient can be studied over a long period of time and when relatives can also be examined. It is sometimes difficult however, to make a firm diagnosis at the time of the first series of examinations because no single laboratory test is diagnostic. As diagnosis of these two well defined disorders becomes more precise, atypical forms of hemolytic disease, both congenital and acquired are more clearly recognized.

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STRESS URINARY INCONTINENCE IN THE FEMALE; A REVIEW OF THE MODERN APPROACH TO THIS PROBLEM

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STRESS incontinence, a common gynecologic symptom, is characterized by the loss of urine through the intact urethra, under conditions which increase the intra-abdominal pressure, such as laughing, coughing, sneezing, lifting or straining. The exact incidence is not readily available, but Goldberger and Davids[18] found that of all patients requiring vaginal plastic surgery, 37.8% suffered from varying degrees of stress incontinence. Kegel[25] reported that 5.5% of women whom he studied exhibited poor bladder control. It is probably more frequent than this, since many women fail to mention this symptom unless questioned about it. Many of these patients withdraw from certain of their social activities for fear of embarrassment instead of seeking relief from their symptom. Indeed, certain women consider incontinence to be an inevitable sequel of childbearing and of advancing age. The condition usually manifests itself late in the childbearing period, or during the menopause, although it may appear at any time.

Stress incontinence is either congenital or acquired. The congenital type may be present from birth. The patients suffering from this type frequently give a history of having a "weak bladder" from early life through adolescence. This is true especially of nulliparous women who develop stress incontinence in later life.

The acquired types may follow obstetric trauma, surgical procedures on the vagina or uterus, or the atrophy which occurs at the menopause.

Anatomy of the Female Urethra and Bladder. The urethra varies in length from 3 to 5 cm. Kennedy[27] found its average length to be between 4.0 and 4.5 cm., and Krantz[31] between 3.0 and 3.6 cm. The internal or vesical orifice of the urethra lies approximately 3 cm. behind the midportion of the symphysis pubis. From its internal orifice, the urethra extends downward and forward so that its external meatus opens in the vestibule of the vagina, about 2 cm. below the base of the clitoris.

In its course from the bladder to the external meatus, the urethra penetrates the three diaphragms of the pelvic outlet: (1) the pubo-vesico-cervical fascia, (2) the urogenital diaphragm, and (3) the pubococcygeus muscle, with its fascia and adjoining musculofascial supporting structures.

Kennedy[30] studied two urethras by serial section. He cut one of them at right angles to the longitudinal axis of the canal. The other was sectioned parallel to the long axis of the urethra, and at right angles to its sagittal plane. His conclusions follow:

"The true sphincter of the urethra is *wholly* composed of the circular smooth muscle surrounding the middle and inner thirds of the urethra, together with their associated longitudinal smooth muscles. When relaxed it may be thought of as a *cylindrical sphincter*. In this 'relaxing' state it prevents any urine escaping from the bladder."

"A study of the urethra with serial sections, concentrating specifically on the intrinsic voluntary muscle fibers, reveals that they compose a single muscle, having an unusual shape and as unusual a function. It has been temporarily named the *muscle of micturition*."

"The muscle of micturition has two origins, possesses its own identity, is entirely independent from and opponent to the levator ani, the bulbocavernosus, the transversus perinei, and the ischiocavernosus muscles, and has two insertions. Two nerves supply it, passing in near its origins, one branch each from the pudendal trunk ascending parallel to the ramus of the pubis. Its function, when contracting, is to distort and open the true or cylindrical sphincter."

Krantz[31] studied 7 urethras in a similar but more detailed manner. He used stains which allowed him to distinguish readily smooth and striated muscles, nerves, collagen, myelin and epithelial surfaces. He concluded the following:

"The lower two-thirds of the urethra traverses the anterior vaginal wall and is inseparable from it.

"The circular muscle of the lower third of the vagina encircles the urethra superiorly and inferiorly.

"The longitudinal smooth muscle of the urethra lies close to the lumen and is continuous with the longitudinal musculature of the bladder.

"The circular muscle of the urethra is of two types:

"Smooth muscle fibers encircle the longitudinal muscle fibers throughout the urethra in a spiral fashion and are continuous with the circular musculature of the bladder.

"Striated muscle fibers which originate from decussating fibers of the ischiocavernosus and bulbocavernosus muscles encircle the middle third of the urethra to form a striated sphincter.

"There is no specific localized smooth muscle sphincter in the urethra. Thus the urethra and bladder are apparently one anatomical unit and function as such.

"The bladder is composed of two closely interlaced layers of smooth muscle, longitudinal and circular.

"The presence of the 'puboprostatic ligaments' in the female has been established."

Krantz attributed his radically different findings to the fact that he had employed a stain which made it possible to differentiate muscle from connective tissue.

Ullery[55], in his recent monograph, summarized the situation as follows: "Three groups of muscles may be described as forming the musculature of micturition: (1) the detrusor vesicae, (2) the 'internal sphincter', and (3) the 'external sphincter'." Summarizing from Ullery's review, the following facts are pertinent to this topic:

1. The muscular wall of the bladder is composed of three layers. These are separated indistinctly into an external longitudinal, a middle circular, and an internal longitudinal layer. These form the detrusor vesicae. For practical purposes, these three layers function as a unit. The circular layer is the most complete and most important. Of the two longitudinal layers, the external is the more important and is thickest in the sagittal plane. There is a thickening of the bladder wall, termed the musculus trigonalis, in the region of the trigone, where it is superimposed on the detrusor vesicae. This area of the bladder is not subject to change when the organ is distended. It is formed from a fan-wise extension of longitudinal muscle fibers from the dorsal walls of each ureter.

2. The "internal sphincter" lies distal to the internal urethral orifice, where the circular fibers of the urethra intermingle with the longitudinal ones. These combined fibers are continuous with the bladder fibers. Although Krantz found no true sphincter in this region, most anatomists and histologists believe that the true sphincteric control of the bladder outlet lies in this area. It is also in this region that Kennedy found his muscle of micturition.

3. The "external sphincter" is formed by decussating fibers of the bulbocavernosus and ischiocavernosus muscles, together with those of the deep transverse perinei muscles. Of great importance also is the supportive fascia of the pubococcygeus muscle which aids in compressing the urethra, and produces a sphincteric effect together with the accessory muscles.

The Physiology of Continence. Kennedy[30] concluded that continence is maintained wholly by the circular smooth muscle surrounding the upper two-thirds of the urethra, together with its longitudinal smooth muscle. "When the muscle of micturition relaxes, the true or cylindrical sphincter exhibits its maximum control and no urine runs out of the bladder. When it contracts completely the cylindrical sphincter is distorted into an oval formation throughout its length. In this 'voiding' state all the urine can escape from the bladder. If complete contraction becomes permanent, complete incontinence exists. Any permanent partial contraction will carry with it a corresponding degree of incontinence."

Krantz[31] believes that the musculature of the urethra and anterior vaginal wall has the following role in micturition: "The urethra and bladder function as a unit. This unit may be likened to a lever system of the first class: the bladder at one end and the external urethral meatus at the other, while the puboprostatic ligaments act as a fulcrum. When the bladder is sufficiently full, sensation is perceived cerebrally through the visceral afferents. Cord reflex with modulation through cerebral control initiates the response for voiding through stimulus in the parasympathetic nervous system. Increase in the intra-abdominal pressure accompanied by contraction of the bladder and urethral smooth muscle results in a reduction of the bladder capacity with concurrent increase in

the intravesical hydrostatic pressure. The urethra shortens through contraction of its longitudinal fibers, and, because the longitudinal fibers are closer to the lumen, there is concentric displacement of the circular fibers of the urethra which are also in a state of tonus. As a result, the lumen is opened, allowing urine to escape. Closure of the urethra, making the individual continent, is achieved through the tonic contraction of the smooth circular muscle. The longitudinal muscle may be said to be antagonistic to the circular smooth muscle of the urethra. If cessation of voiding be initiated on a voluntary basis, it is brought about through the closure of the urethral lumen by the striated sphincter in the region of the middle third. Since this sphincter is an integral part of the bulbocavernosus and ischiocavernosus muscles, it has voluntary (spinal) innervation. During voluntary control or on contraction of the striated sphincter, the urethra rises in and upward into the vagina. The role played by the sympathetic nervous system is not clearly understood; however, from the evidence now present in the literature, it is relegated to a minor role."

The studies by Krantz indicate that two anatomic defects produce incontinence: (1) inadequacy of the support and fixation provided by the puboprostatic ligaments, and (2) inadequacy of the voluntary and smooth circular muscles of the urethra.

Berkow[9] recently proposed an added factor in the maintenance of bladder control: the corpus spongeosum of the urethral submucosa, which exerting a "washer" effect, aids in reinforcing the urethral sphincters. This theory is supported by the clinical fact that postmenopausal patients, who are prone to incontinence, have greatly decreased urethral vascularity and turgidity.

Radiographic Observations. Clinicians have studied incontinent women by the use of roentgenograms, and agree on the findings described below. Kennedy[27], employing a urethral balloon filled by a contrast medium, showed that the proximal portion of the urethral sphincter was stronger than the external portion. He found that the mid-urethral sphincter was the strongest of the three. In the normal urethra, the proximal portion of the involuntary sphincter was found to dilate at 30 to 40 cm. of water pressure, while the voluntary or middle sphincter did not do so until the pressure was raised to 80 cm. From studies upon his incontinent patients he concluded, that, when the voluntary sphincter dilates at 30 cm. of water pressure, it is justifiable to make a diagnosis of incontinence. Similar studies by Barnes[5,6] confirmed the findings of Kennedy. He felt that incontinence resulted from two factors, increased intravesical pressure, and decreased urethral resistance, one or both of which could be operative in producing the symptom. By cystometric curve studies, he demonstrated a definitely increased intracystic pressure when subjects were changed from the recumbent to the standing position. Barnes used a balloon containing 90% sodium iodide at 35 cm. water pressure to study urethral resistance. He found that in the normal individual, using this pressure, urethral resistance displaced most of the fluid to the ends of the balloon outside the urethra, while incompetent urethras failed to displace this fluid and dilated along their length. In addition he passed a watch chain along the urethra via a catheter to study the effect on the contour and curve of the urethra. With this he demonstrated funneling of the bladder neck in patients with stress incontinence.

In 1949, Muellner[37] studied the location of the bladder by the use of a radio-opaque medium. He found that when the patient was in the recumbent

position, the normal bladder base lay above the symphysis. When the patient stood up, only slight descent of the bladder base occurred. In patients who were incontinent, he found pronounced descent of the bladder base, and a pointing or a teat-like outpouching of the region of the vesical neck, either on stress or on standing. He concluded that the internal portion of the urethral sphincter and the pubococcygei muscles, which he felt formed the mid-sphincter, are the vital factors in urinary control. Exertional incontinence results from the failure of these muscles to support the bladder neck. Comparable radiographic studies were conducted pre- and postoperatively by Marchetti[34] with similar results.

Ball, Fulkerson and Douglas[3] presented a method which demonstrated the position of the bladder neck in relation to the inferior border of the symphysis. They covered a Foley catheter with a sheath so that it could be filled with contrast fluid. The catheter was inserted into the bladder so that the sheath extended through the urethra. The bladder was filled with 5% sodium iodide, while the sheath and the Foley bulb received 30% sodium iodide for the purpose of contrast. Lateral roentgenograms were made with the patient sitting and standing. In each of these two positions, a film was taken before the patient tried to void; a second film while she made an effort to void; and a third film when the patient tried to constrict the urethral muscles. In each lateral view of the pelvis, they drew a line projecting through the superior and inferior edges of the symphysis. They defined this line as the lateral bore of the symphysis. In the average nullipara, the bladder neck was found to lie 1.8 cm. behind this line, while the external meatus lay anterior to it. In the case of patients with stress incontinence, the bladder neck was found to be anterior to the lateral bore. Thus the bladder neck had been displaced both downward and forward. Funneling of the bladder neck also was observed. The inner third of the urethra exhibited an accordion effect with anterior displacement of the external meatus. Films of patients with congenital incontinence revealed the same relationships as did the films of those who suffered from incontinence due to the trauma of parturition.

Jeffcoate and Roberts[2,21] studied intravesical pressure in relation to incontinence. They found that the normal resting intravesical pressure is 10 cm. of water, and that under stress it rises to between 50 and 75 cm. They made the following observations with regard to stress incontinence[21]:

"When the patient is asked to cough, incontinence occurs at any time, irrespective of the amount of fluid in the bladder."

"The more troublesome the symptom, the lower the pressure required to produce incontinence."

"Many patients remain dry if the intravesical pressure is slowly and gradually raised to a pressure as high as 70 cm. of water, whereas a *sudden* rise to half this pressure causes the escape of urine."

Jeffcoate and Roberts also studied the relationship between the urethra and bladder by the means of lateral roentgenograms. They found that the normal bladder base lies parallel to, and just above a line drawn from the lower edge of the symphysis to the fifth sacral vertebra. The angles formed by the junction of the urethra and bladder, both anteriorly and posteriorly, were well defined. In the normal individual, micturition obliterated the posterior urethrovesical angle, but this did not occur with stress. When patients

with marked uterine prolapse make straining efforts, the urethra and bladder are forced downward. The fact that these patients are not always incontinent under these conditions is explained by the maintenance of a normal posterior urethrovesical relationship. "Irrespective of the degree of descent, the angles of the urethrovesical junction remain clearly defined[21]." With stress incontinence "the most characteristic change is the loss of the posterior urethrovesical angle so that the trigone and urethra tend to come into line"[21]. The standard anterior colporrhaphy may not cure incontinence on account of its failure to correct this posterior urethrovesical angle.

Hodgkinson[20], studied urethrovesical relationships employing the metallic, bead chain technique. In incontinent patients under stress, he found a downward, vertical thrust of the bladder and its contents, so that as a result, the internal sphincter became the region of maximal hydrostatic pressure.

Differential Diagnosis of Stress Incontinence. The presence of stress incontinence may be suspected from a good history, but its diagnosis may be uncertain solely on this basis. The involuntary escape of urine under stress, from a partially-filled bladder through an intact urethra, must be differentiated from many similar conditions. Failure of incontinence to respond to the usual operative procedures is often due to the failure to establish the exact type of incontinence.

The patient who presents herself with symptoms suggestive of stress incontinence requires thorough study during which all other conditions that may produce incontinence are considered. Some of these conditions follow:

1. Congenital anomalies of the urinary tract may cause intermittent urinary leakage. Among these are ectopic ureters, extrophy of the bladder, diverticula and strictures of the urethra, congenital valves in the proximal urethra, and faulty development of the musculature about the bladder neck and urethra. The incontinence associated with these will often begin early in childhood. The last named, however, which is frequently associated with spina bifida, may not produce symptoms until later in adult life.

2. Lesions of the nervous system, with so-called "cord bladder", may result in paradoxical incontinence with intermittent or constant dribbling from an overdistended viscus. The lesions most commonly associated with this type of incontinence are tabes dorsalis, multiple sclerosis, benign or malignant spinal cord tumors, transverse myelitis, and Pott's disease of the spine.

3. Intrinsic lesions of the bladder neck and urethra which interfere with normal sphincter mechanisms are mainly tumors, calculi, suburethral cysts, and trigonal hernias. Their action may be purely mechanical, or they may give rise to inflammatory changes in the bladder or urethra, which in turn result in extreme urgency and frequency which simulate stress incontinence. Chronic inflammation of the urethral glands is considered by Hoch[19] to be one of the commonest causes of incontinence in women. This was also stressed by Rashbaum and Mandelbaum[43], who considered one of the prominent causes of incontinence to be papillary posterior urethritis, due to infection of the glands near the vesical orifice. These inflammatory conditions produce extreme urgency and frequency of urination, to which the term "false incontinence" has been applied. These may produce enuresis in childhood; they may appear for the first time after menstruation has begun; or they may appear initially after

the onset of the menopause on account of the atrophy that accompanies endocrinal deficiency.

4. Extrinsic causes include the following: cervical or corporeal uterine growths, inflammatory or neoplastic adnexal masses, sigmoid malignancy or diverticulitis. They produce urgency that simulates incontinence through vesical distortion or pressure to the point of limited capacity. They may also directly involve the bladder or urethral wall and produce marked vesical irritability.

5. Fistulas of the urinary tract, resulting from obstetrical or surgical injuries, malignancy or irradiation, with intermittent urinary leakage, may at times imitate stress incontinence. Such fistulas may escape detection upon routine inspection. A history of previous trauma, and a careful study by means of urography and intravesical solutions of dye, will permit accurate localization.

Study of the Patient with Stress Incontinence. The study should begin with a complete history, allowing the patient to describe her symptoms in her own terms. She may complain of other symptoms such as vulvar pruritis or irritation, but not of the incontinence itself. An effort should be made to determine the circumstances under which urine is lost, the duration and severity of her symptoms, and any recent changes in symptomatology. The existence of a "weak bladder" at any previous time should be ascertained, as well as the history of antecedent trauma or infection. This should be followed by a general physical examination with emphasis on possible neurologic deficiencies. A pelvic examination with visualization of the cervix is then carried out. At this time the patient is asked to strain down so that the degree of pelvic relaxation is evaluated.

The following diagnostic measures are important in proving the existence of true stress incontinence:

1. URINALYSIS. A catheterized specimen should be obtained for chemical analysis, microscopic examination, and culture. Voided specimens are useless for this purpose.

2. ENDOSCOPIC EXAMINATION. Thorough visualization of the bladder and urethra is essential in order to rule out the presence of any of the intrinsic lesions that have been mentioned above, and to exclude the existence of extrinsic lesions that might affect bladder function. In addition, the use of a dye such as indigo-carmine, by renal excretion or bladder instillation, may be helpful in localizing a high or obscure fistula.

3. INTRAVENOUS UROGRAPHY. This will aid in detecting lesions of the upper urinary tract such as ectopic ureters. It can also serve to demonstrate urethral sphincter incompetence according to the technique of Briney and Hodes[10].

4. CYSTOMETROGRAM. This measures the bladder capacity and helps to rule out the possible existence of neurological causes for urinary incontinence.

5. CYSTOGRAPHY AND CYSTO-URETHROGRAPHY. The techniques described by Ball[3] and Jeffcoate[21] are invaluable in establishing the diagnosis of stress incontinence. Their methods of study are important adjuncts not only in diagnosis, but also in determining the extent to which it is necessary to elevate the urethra and vesical neck. The lateral views of the urethra and bladder are of particular importance, and postoperative examinations are helpful in evaluating the results of the procedure.

6. URETHRAL ELEVATION TEST. This maneuver aids in confirming the diagnosis of stress incontinence, and in predicting the result to be expected from urethral and vesical neck reposition. Bonney supported the urethra and

bladder neck by the use of digital pressure on either side of the urethra. By this means, the involuntary escape of urine from the bladder could be prevented. The use of fingers might be misleading because of their breadth. Read elevated the vesical neck with the ends of long thumb forceps. Marshall[36] filled the bladder with 250 cc. of saline solution, and had the patient demonstrate her incontinence by coughing and straining in the lithotomy and standing positions. He injected procaine into the anterior vaginal wall at the level of the interureteric ridge. He then grasped this area with an Allis forceps, and pushed it upward toward the umbilicus. If the patient retained the fluid in her bladder on coughing and straining, he regarded the test as positive, indicating a good likelihood that she could be cured by suprapubic vesicourethral suspension.

7. SPECIAL VAGINAL EXAMINATION. Examination of the urethra and bladder while the patient stands and bears down, with one foot on a low stool, often reveals a relaxation which might not be apparent with the patient in the lithotomy position.

Methods of Treatment. The current treatment of stress incontinence ranges from the conservative, non-operative treatment on the one hand, to the radical operative treatment on the other.

NON-OPERATIVE TREATMENT. 1. Physiologic treatment, directed toward development and re-establishment of tone and function of the pubococcygei muscles, has been advocated by Kegel and his associates[22,23,24,25]. Their treatment is based on the clinical finding that many patients exhibit atrophy of the voluntary muscles in the region of the vesical neck. They place emphasis upon the need to strengthen and increase the tone of these muscles which have been damaged during parturition, or in which tone was always poor. They also found that between 30 and 40% of all women lack normal muscular function, and have no awareness, or only a limited awareness, of their ability to contract these muscles voluntarily. Evaluation of function is made with the patient in the lithotomy position and with the examining finger palpating the lateral regions of the middle third of the vagina. The patient is asked to "pull up" or "hold" as if she were attempting to control her bowels or hold her urine. Approximately one woman in three is unable to appreciate the fact that she can do this voluntarily. The strength of the contractions which she can produce are measured by means of a pneumatic resistance chamber, called the perineometer. This fits the vagina and is connected to a manometer by a piece of rubber tubing. The average patient produced pressures varying between 30 and 60 mm. of mercury, while those who were unaware of their ability to contract these muscles, produced little or no increase in pressure.

Kegel has applied these exercises in the treatment of many types of pelvic floor inadequacy. The method of exercising these muscles is explained to the patient, and she is advised to perform them for a period of 20 minutes 3 times a day. The perineometer is a useful instrument for checking the progress of the patient. In those patients who were unaware of the fact that the pubococcygeus muscle could be contracted, the perineometer offered a visual means of observing the action of this muscle.

Jones and Kegel[22] treated 117 patients with stress incontinence by this method. Of this number 69% showed improvement. Six per cent showed good results which were followed by relapse when they failed to continue their exercises. Twenty-five per cent

showed no improvement. They also treated 37 patients who were still incontinent following previous vaginal surgery. Seventy-one per cent of these were relieved by exercises.

Definite improvement usually followed 6 weeks of exercise, but the ultimate gain was not achieved for at least 6 months. This treatment is not designed to replace surgery when this is indicated, as, for example, in cases of descensus, cystocele or rectocele. However, its pre- and postoperative use improves the results in patients who are treated surgically, and in the event that the patient will not tolerate or consent to operation, it serves as an excellent alternative procedure.

Although Wharton[56] does not feel that the perineometer is essential to the performance of this treatment, he believes these exercises are an important adjunct in the management of stress incontinence. He lists the following indications: poor operative risks, surgical failures following operation for stress incontinence, stress incontinence developing after other gynecologic operations, and as supplementary treatment in urologic conditions associated with stress incontinence.

2. Urologic therapy is undertaken whenever it is necessary to eradicate local foci of chronic infection. Rashbaum[43] dilates the urethra at weekly intervals and applies a 5% solution of silver nitrate to the trigone, bladder neck and urethra. He supplements this with exercises consisting of repeated starting and stopping of the urinary stream, and with the administration of ephedrine for its effect upon the sympathetic nervous system. Of 68 patients whom he treated with this plan of therapy, 40% were cured and 41% improved.

3. Hormonal therapy has been suggested by Salmon *et al.*[47] for the purpose of overcoming the atrophy of the genital tissues that follows the menopause. He treated 10 patients with estrogenic hormones. He observed symptomatic improvement of 5 patients within one week. The symptoms of these patients recurred in 6 weeks to 4 months after treatment was stopped.

When the cause of the incontinence cannot be determined, a trial of combined urologic, hormonal, and physiologic therapy is justified before any type of surgical approach is carried out. The adverse effect of prolonged estrogen therapy must be borne in mind, if it is to be used. Conservative therapy may obviate the need for surgery in certain cases.

OPERATIVE TREATMENT. Basically, four types of surgical approach are used in the treatment of stress incontinence: 1. Reconstruction of the vagina (the so-called "standard" type of vaginal operation), 2. Advancement of the urethra. 3. Transplantation of fascia under the urethra (the "sling" operation). 4. Suspension of the urethra and bladder behind the symphysis (the "pin-up" operation).

1. *Vaginal reconstruction.* These operations aim at strengthening the fascia supporting the bladder and urethra, re-establishing the posterior urethrovesical angle, and decreasing the size of the urethral lumen. In 1913, Kelly[26] suggested plication of the fascia at the bladder neck and the posterior urethra. An anterior colporrhaphy was often added to this procedure. In subsequent years, the Kelly operation was modified in many ways to provide additional support. TeLinde[54] modifies the Kelly procedure by denuding the vaginal mucosa covering the posterior part of the urethra. He plicates the fascia under the urethra, placing special emphasis upon the plication in the region of the vesical neck. Kennedy[27,28,29], on the basis of his studies, plicates

the suburethral fascia after separation of the urethra from the pubis. He considers the dissection of the urethra from the pubis to be the most important step in his operation.

These procedures represent the basic types of vaginal surgical approach. On the average, 10 to 20% of patients will experience a recurrence of their symptoms following any one of these types of operation. Counseller[13] recently reported the results of 578 operations for stress incontinence. He utilized three different techniques in the performing of these operations. These were the Kelly, the modified Kelly, and the Kennedy operations. Of 227 patients treated by the Kelly operation, 85% became continent, 5% were improved, and 10% remained unimproved. One hundred and ten patients were subjected to the modified Kelly procedure. Eighty-seven per cent were cured; 7% were improved; 6% were not improved. Following the Kennedy operation on 179 patients, 85% were cured, 8% were improved, and 7% remained unimproved.

Counseller treated a series of women who had had previous vaginal surgery for stress incontinence without relief of their symptoms. He performed the Kelly operation on some, and the Kennedy operation on the others. The Kelly operation was followed by cure in 76% of the patients, improvement in 10%, and failure in 14%. The Kennedy operation was followed by cure in only 48% of the patients, improvement in 22%, and failure in 30%. As a secondary procedure, therefore, the Kelly operation was twice as successful as the Kennedy in this series.

TeLinde[54] performed the Kennedy procedure on 249 patients. He cured 90.3% and improved 5%. Phaneuf *et al.*[42] had a 91% cure rate in 23 patients.

The 90% cure rate, added to the relative ease of performance of the vaginal operations, make these the rational ones with which to undertake surgical treatment of stress incontinence.

2. *Urethral advancement.* Berkow[8], in 1941, described this procedure consisting of elevation of the urethra, with lengthening and narrowing of its lumen, so that its external meatus is advanced close to the base of the clitoris. This operation was first used by Pawlik[40] in 1883, and later by Bonney. Berkow also buttressed the urethra with the pubococcygeal and bulbocavernosus muscles. He reported continence in 17 of 19 patients with follow-up ranging from 2 months to 3 years. Reis and DeCosta[46] used Berkow's technique on 33 patients. They cured 31 of them. They believe that its greatest value is in cases of stress incontinence following obstetric injury, and advise postoperative observation for at least 6 months before a final evaluation of the results is made.

3. *Suburethral fascial transplantation.* This type of surgical treatment, though not of recent origin, is at present the most controversial in the approach to the treatment of stress incontinence. The discussion centers about the need for the "sling" operation as an initial effort, in view of the cure rate following standard vaginal procedures.

In 1910, Goebell[17] elevated the urethra by the use of a sling made of the pyramidalis muscles. Four years later, Frangenheim[15] reported the use of the pyramidalis muscle and its fascia, and at times he used the rectus fascia when the pyramidalis was poor. Stoeckel[50], in 1917, combined the procedures of Goebell and Frangenheim and added to them a vaginal procedure in which he plicated the structures about the vesical neck. The "sling" procedures are generally known by the name Goebell-Frangenheim-

Stoeckel operations, and variations introduced by other surgeons are modifications of this technique.

Three modifications have come into popular usage during the past decade. The first is the Aldridge[1] operation as described in 1942. The second is Studdiford's variation of this procedure[51,52,55], and finally there is the method of Millin and Read[44,45].

The Aldridge procedure consists of dissecting the urethra from the vaginal wall and from the symphysis, to the point at which the abdominal muscles attach to the pubis. The tissue of the inferior surface of the urethra is then plicated, including the region along the posterior surface of the urethrovesical junction, and the anterior vaginal wall is left unsutured. Through a suprapubic abdominal incision, a strip of aponeurosis from both of the external oblique muscles is formed into a sling. The distal ends of these strips of aponeurosis, still attached to their muscular origin, are passed through either rectus muscle, behind the symphysis and into the suburethral space, where they are sutured together. Following this the vaginal wall is closed. To avoid unnecessary changes in the position of the patient, the suprapubic portion of the operation may be performed before the vaginal portion of the operation is undertaken.

Studdiford's modification of the Aldridge operation consists of freeing a single longitudinal strip of rectus fascia and leaving one end still attached to the rectus sheath. The free end of this strip is carried under the urethra and is attached to the opposite rectus muscle. He considers that the use of the rectus fascia offers less chance for weakness or herniation of the abdominal wall than does the use of the fascia of the external oblique muscles.

The procedure of Millin and Read has no vaginal approach. They form aponeurotic slings secured from the sheaths of the oblique and rectus muscles. Each of these is carried through its rectus muscle, under the urethra, and returned to the sheath of the opposite rectus muscle where it is attached. The sling is so fixed that any increase in intra-abdominal pressure is associated with further elevation of the bladder neck. Following this procedure the patient must re-learn to void, because any straining effort will be met by the resistance of the sling. Relaxation of the abdominal muscles is necessary in order to initiate micturition.

The indication for any sling procedure is failure to relieve the incontinence by a standard vaginal procedure. Those who have used the sling procedures extensively do not recommend them as primary efforts or for patients who suffer from uterovaginal prolapse. There is, however, reason to perform the sling operation primarily for patients who suffer from a chronic stress state such as asthma or bronchitis. This also applies to patients whose occupations require standing for long periods, or lifting heavy objects. Paxson[41] recently emphasized these points in his report that compares the various types of surgical treatments for incontinence.

The results obtained by the use of slings are comparable irrespective of the technique employed. Studdiford[53] reported an 86% cure rate in 44 patients by his technique. Read[44], using the Millin-Read technique, reported an absolute cure in 91 of 125 patients, with partial cure in 21, and failure in 13. The majority of his failures were due to insufficient elevation of the bladder neck. Jeffcoate[2], using the Aldridge technique, cured 23 of 25 patients. His studies led him to believe, that

the important factor in relief of stress incontinence, regardless of the procedure, is correction of the posterior urethrovesical angle, rather than mere elevation of the bladder neck. Sloan[49] reported 28 cures in 31 patients by using the Millin-Read procedure.

Other tissues have been used in the "sling" operation. Barnes[7] sutured the round ligaments under the urethra. Shaw[48] excised a wide strip of fascia lata, placed it under the denuded urethra, and passed it through holes in the pubic bone to be sutured in front of the symphysis. The danger of osteomyelitis of the pubis must be considered, although few cases have been reported. O'Sullivan[39] reported on 58 such operations with only 1.7% recurrence of symptoms.

In patients for whom previous standard vaginal procedures have failed to produce relief of symptoms, the sling operation is generally successful, in spite of the scar tissue resulting from the previous operation.

The disadvantage of the sling operation lies in the time requirement, and in the amount of tissue injury that is associated with its performance. These may lead to a greater number of complications. Despite these disadvantages, the sling procedure seems to be justified in selected cases, as a secondary surgical attempt.

4. *Suprapubic vesicourethral suspension.* In 1949, Marshall, Marchetti, and Krantz[36] described the following procedure. This consists of entering the space of Retzius through an abdominal incision and of separating the urethra and bladder from the under surface of the symphysis. The vaginal wall is sutured to the periosteum of the symphysis pubis on either side of the urethra. In the original description of the procedure, the vaginal wall sutures were carried through the urethral wall. Marchetti[35] and others observed much less injury to the urethra if the vaginal wall sutures did not include the wall of the urethra. The lower and lateral portions of the bladder are then sutured to the under surfaces of the rectus muscles. Their first report showed 82% of 44 patients to be cured, 7% significantly improved, and 11% unimproved.

Marchetti[34] studied his patients postoperatively with roentgenograms. He found that the most striking postoperative change was elevation of the bladder. In addition, the bladder neck and urethra were found to lie in close proximity to the posterior surface of the symphysis without distortion of their relationships. Roberts[2] studied 3 patients postoperatively by means of lateral cystourethrograms. He concluded that the success of the operation was due not only to elevation of the urethrovesical junction, but also to a restoration of the normal posterior urethrovesical angle.

Other operators have reported equal success with this procedure. Chalmers[11,12] cured 27 of 31 patients; Doolittle[14] had complete success in 6 patients; Gillam *et al.*[16] reported only one failure in 20 patients.

In 1952, Ball[4] utilized the Marchetti type of procedure, but modified it by adding a vaginal approach to the operative field. He reported 94% cures in 69 patients, with follow-up from 2 months to 3 years. The failures became evident so soon after operation, that he feels confident that those patients who are now relieved of their symptoms, will have no recurrence.

5. *Other procedures.* Mulvany[38], in 1951, described the technique of vesicourethrolysis, based upon the theory that a sphincter must be free to maintain normal function. After complete circumferential dissection, the urethra is left free without the benefit of fixation or plication. In 58 patients, con-

tinence was restored without a failure. Lennon[32,33] found similar success with this procedure, but added support from below by plication.

Summary. Interest in stress incontinence has been intensified by recent anatomic and radiographic studies of the female bladder and urethra. Both have added much to our knowledge of the physiology of micturition, and the pathology of urethral incompetence.

The basic defect is the loss of integrity of the musculo-fascial supports of the urethra and bladder. The exact origin and identity of these supports is an unsettled matter. The methods by which these supports may be re-established or rehabilitated are also controversial.

Not controversial, however, is the fact that, the first step in the management of incontinence must be the elimination of many other female urinary tract conditions that produce symptoms similar to those of stress incontinence.

When the incontinence is proved to be due to stress, the choice of treatment depends upon the cooperation of the patient, the extent of the accompanying relaxation, and the history of previous conservative management or surgical therapy.

Non-operative measures may relieve stress incontinence, or they may serve as valuable pre- and postoperative assets to enhance the ultimate functional result.

The literature indicates that, should operation be necessary, the first surgical approach should invoke one of the standard vaginal procedures. For the small number of failures, one of the more complicated operations is in order.

At present, the choice between urethral advancement, suprapubic suspension and suburethral fascial transplantation is a matter of personal preference. In time, with the accumulation of both pre- and postoperative data, specific indications for each of these procedures should be developed, with general improvement in the ultimate functional results.

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THE IMPORTANCE OF TRYPSIN IN INFANCY AND CHILDHOOD

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THE purpose of this paper is to explore some of the more recent contributions to the study of digestive secretions in infancy and childhood as a supplement to previous communications[166,167]. Our attention is primarily directed toward emphasizing the more basic phenomena related to the role of trypsin in the physiology of the child. The literature covering new developments in trypsin during the last 10 years has been surveyed. The presentation has been divided into two parts: I. **Basic Considerations** and II. **Clinical Implications** (Part II will appear in June, 1954).

I. Basic Considerations.

Each decade brings new approaches to the study of the nutritional disorders and the enzyme responses in physiologic and pathologic conditions. The integration of enzymes with a variety of substances has been observed lately: of the vitamins, four are now known to be essential component parts of certain enzymes, so that a lack of either nicotinamide, riboflavine, thiamine or pyridoxine causes a lack of the corresponding enzyme. More and more drugs and chemical compounds are found to produce their characteristic effects by inhibiting or activating a particular component in a metabolic enzyme system. The hormones have been slower to reveal their relationship to enzymes, but a start has been made by Cori's discovery (1945) that a hormone from the anterior pituitary inhibits the enzyme hexokinase, essential to carbohydrate metabolism. In 1947 and 1948, the inhibiting effects of estrogens on succinic dehydrogenase and malic dehydrogenase were described respectively by Case and Dickens[28] and Erway and associates[47].

The last decade has also been one of rapid advances in forging new tools for more specific diagnostic tests and developing new methods for evaluating enzyme activity. These studies,

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when considered in connection with other investigations, have helped to introduce a sounder basis for experimental work. More and more investigators interested in pediatrics are devoting their time and effort to the investigation of the digestive enzymes in childhood[13,26,30,34,40,46,49,59,60,85,88,103,107,109,110,112,141,155,156,162].

The difficulties which attend the clinical estimations of the digestive enzymes in childhood may be outlined as follows: 1) Technical difficulties in procuring specimens from the small intestine with a minimum of resistance and excitement from young patients. 2) The scanty output of the digestive secretions under resting test conditions. 3) The inconstancy of the secretory pattern from one individual to another and in the same individual from one day to the next. Related to this difficulty is the effect of emotion or excitement upon gastrointestinal functioning, particularly in infants and children. 4) Difficulties in obtaining pancreatic or other localized secretions uncontaminated by variable amounts of bile, gastric juice, or secretions from other areas. 5) The semi-quantitative nature of the methods conveniently available for the enzymatic components, so that the results of estimations have to be expressed in units of low precision.

DEFINITIONS. A *catalyst* may be defined as a substance which alters the speed of a chemical reaction without appearing in the final products of the reaction. An *enzyme* is a specific biochemical catalyst produced by the living cell, though independent of the living cell in its action. Enzymes control biochemical reactions only by altering their velocities. They are almost always protein in nature, and have high molecular weights.

A *co-enzyme* is a thermostable, dialyzable prosthetic group, organic in nature, which is attached to the protein for the final highly active enzyme. Not all prosthetic groups are coenzymes, and not all enzymes have prosthetic groups. It is accepted that the difference in dialyzability is an important distinction between vitamins and enzymes. Nevertheless, certain vitamins have been identified as coenzymes.

The term *protease* or proteolytic enzyme refers in the broadest sense to all enzymes capable of hydrolyzing peptide bonds. Two general classes of proteases have been differentiated: *proteinases* (endopeptidases) and *peptidases* (exopeptidases). Tentative attempts have been made to classify the proteases in terms of (*a*) their specificity of action on synthetic substrates, (*b*) their animal or plant origin, (*c*) their extracellular or intracellular action, (*d*) their optimal hydrogen ion concentrations, and (*e*) their activation or inactivation by various compounds. Unlike most enzymes, the proteolytic enzymes appear to lack prosthetic groups.

The *extracellular proteinases* include pepsin, trypsin, chymotrypsin, uropepsin and some yeast, mold and bacterial proteases. The *intracellular proteinases* comprise (*a*) those of animal origin called cathepsins; (*b*) those of plant origin including papain (from the papaya plant), ficin (from figs) and bromelin (from pineapple); (*c*) most of those from yeast and molds, and (*d*) most bacterial proteases. Leukoprotease, the proteolytic enzyme of white blood cells, is classified with the extracellular enzymes because its properties are similar to those of trypsin. The proteinases are associated with the hydrolysis of central peptide bonds within the molecule, and are therefore sometimes grouped together under the heading of *endopeptidases*.

The *exopeptidases* act only upon the peptide linkages that are adjacent to

the end of the protein molecule: the so-called "free terminal polar groups", such as amino and carboxyl groups. There is carboxypeptidase which removes terminal units with free carboxyl groups, aminopeptidase which splits off units with free amino groups, and dipeptidase which splits dipeptides into amino acids.

Further subclassification of the proteinases falling under these generic terms must be empirical and has severe limitations. However, the recent identification of proteinases as crystalline proteins, and the development of pure synthetic polypeptide substrates for testing, are steps toward better characterization of certain enzymes by their chemical specificity in terms of sites of action within the substrate molecule. The ultimate aim of this approach is to deduce, from the structure of the substrate, the complementary structure on the enzyme surface. This approach has not been restricted to proteolytic enzymes. Its usefulness has, perhaps, been most keenly felt in the field of immunochemistry, as exemplified by the work of Landsteiner on the specificity of serologic reactions.

TRYPSIN. *Historical Background.* The importance of the study of pancreatic enzyme activity in the duodenal content has been recognized by pediatricians for almost half a century. It is of historical interest that the hydrolysis of amino acid esters by crude pancreatic extracts was first demonstrated by Otto Warburg in 1905[158] and was used for the enzymatic resolution of racemic mixtures of amino acids. It should be noted that the unequivocal proof of a specific esterase activity of trypsin is of recent origin (1948)[144].

In 1912, Hahn and Lust[73] reviewed the previous contradictory literature on the presence of trypsin in stools. These authors secured evidence indicative of tryptic activity in all infants studied, when casein was used as a substrate, regardless of the pH of the stool. They also called attention to the finding that adults have less proteolytic activity in the stool than infants.

Since the basic work, in 1912, of Hess[77] who investigated the digestive enzyme activity not only of normal infants and children but also of those with acute nutritional disturbances, marasmus and certain febrile diseases, the procedures for identifying fibrocystic disease of the pancreas from the various other conditions included in the celiac syndrome evolved slowly over the years 1913 to 1938. Lukacs[108] examined 231 children for the presence of trypsin and observed that the stools of children with infections and nutritional disturbances have decreased trypsin, and curiously that children with rickets have from 4 to 10 times the normal amount. Important data concerning pancreatic proteases in early life were also obtained by the observations of Klumpp and Neale[91] and by Siwe[147]. This pioneering work culminated in the recognition of the diagnostic criteria for fibrocystic disease of the pancreas by Dorothy Andersen in 1938[5].

On the chemical side, the modern history of the pancreatic protease began in 1931 when Northrop and Kunitz[127] from the Rockefeller Institute for Medical Research announced the crystallization of trypsin. Within 7 years, these investigators isolated in crystalline form *trypsinogen* which is the inactive precursor of trypsin, *pancreatic trypsin inhibitor* and another proteolytic enzyme termed *chymotrypsin.* In the last 10 years considerable attention has been given to serum antitrypsin activity and to diverse trypsin inhibitors from various sources. Since 1951, a crystalline preparation of trypsin has been available commercially for clinical purposes. It has taken

just about 50 years to reach the present position where time-honored theories are being reinterpreted in the light of critical use of pure laboratory substances and standardized controls.

General Physical and Chemical Properties. Crystalline trypsin was first obtained from the fraction of "commercial trypsin" which was soluble in quarter saturated ammonium sulfate, but insoluble in half saturated ammonium sulfate. When a cold saturated solution of this fraction in ammonium sulfate was warmed slowly to 25° C., square platelets of trypsin appeared.

One hundred beef pancreas glands have been found to yield 64 gm. of crystalline trypsinogen which can be activated to 35 gm. of crystalline trypsin. These weights refer to filter cakes dried by vacuum filtration.

Crystalline trypsin is essentially homogenous by the usual tests. Its molecular weight derived from the Stokes-Einstein law approximates 34,000. Taken in conjunction with the actual molecular weight, its elementary analysis by weight per cent[126] yields these empirical values: C:50.0, H:7.20, N:14.9, S:1.10, P:0. Trypsin has never been shown to contain any elements other than would be derived from amino acid residues. Its composition is that of a typical protein, with no evidence of any kind for the presence of a prosthetic group.

Many erroneous experimental results sometimes taken as the basis of far-reaching hypotheses have been due simply to uncontrolled changes in the pH of mixtures used for the determination. It is of importance that the pH region of maximum stability may or may not coincide with the region of maximum activity. In the case of trypsin, the isoelectric point (range of maximum stability) is pH 7.5. The hydrogen ion concentration for optimum activity has been found to be at pH 8[124].

A remarkable physical property of trypsin is the observation by Mellanby and Woolley[114] that solutions of crystalline trypsin in dilute hydrochloric acid withstand heating to at least 90° C. This peculiarity is a good example of reversible protein denaturation. However, the process is rendered irreversible when a neutral salt is added to the hot solution. Anson and Mirsky[8] also found that in 0.1 N hydrochloric acid, trypsin is almost completely unaltered at 40° C., but is almost completely denatured above 50° C.

The stability behavior of trypsin is further complicated by the fact that on the acid side of pH 2.0 the protein is irreversibly transformed into an acid-denatured protein. As the pH is increased from 2 to 9 the inactive protein which is formed is further digested by the active enzyme. From pH 9 to 12 the processes of inactivation and hydrolysis of the inactive protein are accompanied by alkali denaturation, and at pH 13 the latter reaction predominates.

Specificity. The relation between a given enzyme preparation and a simple organic reactant, whether a definite group of organic substances or a single substance, is known as enzyme specificity. It is often found that similar enzymes from different organs of the same animal, or from different species, will catalyze the same reaction of the same substrate at different rates. The terms organ specificity and species specificity are then employed. The difference in the specificity of proteinases has been shown to be independent of the size of the peptide chain and dependent only on the environment of the linkage. Each enzyme has rather rigid requirements for the amino acid configuration adjacent to the linkage it will split. Trypsin can hydrolyze many types of protein but appears to possess a more specific action on denatured

proteins. Trypsin will attack the amide linkages of arginine and of lysine compounds such as hippuryl-l-argininamide, hippuryl-l-lysinamide and benzoyl-l-lysinamide[78,79]. In each instance, ammonia is liberated. All these compounds possess a free basic ϵ-amino or δ-guanido but no free carboxyl group. If the basic group is suppressed, no tryptic action occurs. Obviously, these substrates for trypsin correspond to what exists in the interior of the peptide chains of proteins where the guanido group of arginine and the ϵ-amino group of lysine are free. The specificity is apparently fairly narrow, since the analogous histidine compounds are not hydrolyzed by trypsin.

The minimum structural requirements for trypsin substrates may be summarized as follows[123]: 1) A positive charge separated from the susceptible bond by a chain not shorter than that of arginine or lysine. 2) A polar group in the α-position to the carbonyl carbon atom of the susceptible bond. Although all polar groups studied have been uncharged it appears that a negative charge at this point would be unfavorable for hydrolysis. 3) A susceptible peptide, amide or ester bond.

Activators and Inhibitors. Inactivation of enzymes, probably of the same type as heat-inactivation, can be caused also by vigorous agitation of solution, by applying high pressure, by radiation and by many chemical reagents. The ultraviolet light gives rise to excitation only of the exposed molecules, whereas the main effect of Roentgen-rays is to cause ionization through loss of electrons. Such an ionization, of course, is much more effective in initiating a chemical change than a mere change in the molecular relationship. Bier and Nord[14] have shown the inactivating effect of electron bombardment on trypsin. This influence is enhanced by increasing the acidity of the enzyme solution. Calcium ions have no protective effect on the inactivation of trypsin caused by electrons, although under normal conditions, these ions stabilize the enzyme. It was concluded from this study that an increased stability of trypsin under normal conditions has no correlation with the effects of ionizing radiation on the enzyme.

Much systematic work has been done in an attempt to find and isolate inhibitors of trypsin[2-4,9,16-18,25,27,33,37,38,43,58,61,62,83,89,90,102,104,105,113,116,118,146,148,149,152,157,159,163]. These studies emphasize the importance of oxidation-reduction in the control of the activity of trypsin. Reducing agents, including several biologically active thio-sulfhydryl compounds (cysteine, sodium thioglycollate, H-S glutathione, hydrogen sulfide), ascorbic acid and sodium cyanide, are known to antagonize strongly the activity of trypsin. For each reductant the degree of inhibition is proportional to the reducing capacity of the medium. The inactivation of trypsin by thiols is marked by the appearance of sulfhydryl[128]. The activity of trypsin is also suppressed to a variable degree by the following substances: serum, plasma, several heavy metals and dyes, unsaturated fatty acids, heparin, lecithin, quinine and urea salts, glycerol and triglycerides, and some alkaloids. Trypsin inhibitor from egg white and tryptic digests of proteins also exert a strong inhibitory influence.

Rothen[136] found that trypsin inactivates multilayers of bovine albumin deposited on a slide through intervening "blankets" of barium stearate. The thickness of the blanket necessary to prevent enzyme action was dependent upon the number and mode of deposition of the underlying layers of bovine albumin. Hanig, quoted by Neurath and Schwert[123], demonstrated that when solutions of trypsin and hemo-

globin are separated by thin collodion membranes which are impermeable to these substances, tyrosine is liberated from the hemoglobin. This enzymatic action can be prevented at a critical membrane thickness. It is difficult however, to reconcile enzymatic action at a distance with the kinetics and the very high substrate specificity exhibited by these enzymes. This high specificity postulates that the long-range forces arising at the enzyme surface are nondispersive in character.

Several trypsin inhibitors have been isolated from biologic material in crystalline form. (1) Pancreatic extracts contain a polypeptide of about 6000 molecular weight which was crystallized by Kunitz and Northrop[99] and found to have specific inhibitory power against trypsin. The inhibitor reacts slowly with trypsin to form a compound containing one molecule each of trypsin and inhibitor; this compound dissociates in acid solution (pH 1.0) and is reformed on neutralization. (2) A trypsin inhibitor found in soy beans was obtained as a crystalline protein by Kunitz[97]. This substance is readily digested by trypsin when denatured. The native protein has a molecular weight of about 24,000 and an isoelectric point at pH 4.5. (3) Tauber and coworkers[153,154] described the isolation from crude lima bean of a crystalline inhibitor. The property of being a heat-stable globulin helps to differentiate it from the soybean inhibitor of Kunitz. (4) In 1951, Laskowski[101,102] reported the crystallization of a stoichiometric trypsin inhibitor from bovine colostrum. This crystalline inhibitor, when compared to the trypsin inhibitor of Kunitz-Northrop, revealed several similar properties. However, the molecular weight of this substance was found to be approximately 89,000, possibly indicating the formation of a trimer. The isoelectric point of the pancreatic inhibitor is 8.7, but that of the colostrum inhibitor is 4.2. It is activated by 2.3 gamma of trypsin.

Noncrystalline antitryptic factors from egg white and various legume seeds (jack bean, lentil, velvet bean, horse bean and black eyed pea) have been extensively investigated[98,99], particularly with reference to the effect of these substances on nutrition. The presence of these inhibitors can interfere with the digestion within the human gastrointestinal tract of these respective foods. No correlation has been established between the effects of autoclaving on the trypsin inhibitor and hence on the nutritive values. The exact mechanism by which these inactivate the pancreatic juice is not yet clearly understood.

Mild oxidants moderately increase the activity of trypsin, whereas those in high concentrations may inhibit its action completely. Other substances including Fe^{++}, Ca^{++}, Mg^{++}, and Ag^{++} have been found to influence favorably the activity of trypsin.

Determination of Trypsin Activity. The need for analytic methods for accurate quantitative determinations of the changes in the protease titer of body fluids associated with various physiologic and pathologic states has been realized by all investigators. During the last decade, clinicians as well as research men have striven to find more specific techniques or to modify those used in the past[1,23,24,35,50,56,57 59, 64,68,85,86,119,121,137,142 143,145].

The desire to obtain substrates as homogenous as possible, to maintain constant the buffer capacity of the mediums during the enzyme-catalyzed hydrolysis, and to secure precision in the end-point readings, are responsible for the wide variety of techniques suggested.

To Northrop[125] goes the credit for the introduction of a valuable quantita-

tive method designed to measure hydrolysis rates of specific substrates by proteolytic enzymes. The concentration of the enzyme is deemed proportional to its activity expressed in arbitrary units. The number of units per gram of the enzyme is a measure of the purity of the preparation. Northrop studied the proteolytic action of trypsin on casein. The results were expressed in terms of milligrams of tyrosine liberated from casein substrate. This technique was adopted by Anson and Mirsky with hemoglobin as a substrate[7] and was adapted to the Evelyn colorimeter by Kohn and associates[92]. The tryptic activity was expressed for convenience in units, that is, milligrams of tyrosine liberated times 1000. In 1943, Riggs and Stadie[131] described a photoelectric method for the determination of pepsin in the gastric juice. The activity of the proteinase on the egg white substrate was measured by a decrease in the turbidity of the mixture. However samples containing more than traces of coloring matter such as bile or blood interfered with the reading of the end-point. Without sacrificing the main advantages of this procedure Friedman[57] used a suspension of coagulated egg as a substrate for the determination of trypsin activity. The final dilution of this suspension contained 1 mg. of nitrogen per cc. The end point was determined by the opacity of the mixture against ruled black lines. During the same period, Ferguson[53] and Müllertz[121] described a fibrinogenolytic method for measuring blood proteases and protease inhibitors.

In 1949, Waldvogel[157] devised a special colorimetric method in which a soluble chromophoric protein substrate, sulfanilamide azocasein, is employed. The increase in color in a trichloracetic acid filtrate of the digestion mixture is a function of enzyme activity. The enzymatic hydrolysis satisfies the condition for a monomolecular reaction and permits activity to be expressed in terms of the velocity constant. A definite advantage of this method over many in which suspensions of insoluble substrates are employed is that the digestion is not influenced by particle size.

A potentiometric formaldehyde titration has been recently proposed by Iselin and Niemann as quoted by Neurath *et al.*[123]. Their buffer, a monovalent tris (hydroxymethyl) aminomethane hydrochloride buffer (pH=8.1), is distinguished by an excellent buffer capacity within the pH range of enzymatic measurement of trypsin, and does not participate in the reaction between chymotrypsin and nicotinyl-L-tryptophanamide. A similar technique based upon the use of primary and secondary amine buffer system and a potentiometric formol titration has been devised by Wirts and coworkers[165]. In order to obtain accurate results, the end-point of the titration must be determined coincidentally with the extent of hydrolysis.

In 1953, Gowenlock[65] described a rapid and reproducible method for the estimation of tryptic activity in duodenal contents. This is an adaptation of Anson's and Mirsky's method. A feature is the provision for a substrate of denatured human serum protein which gives accurate end-point reading even in the presence of contaminants.

Lately, an increasing number of investigators have devoted their attention to a more simple and clinically applicable analysis based on the digestion of gelatin, in tubes or on photographic film. Because of its current widespread popularity this approach is described at length in another section of this report.

For details of the procedures used in the quantitative analysis of proteins, peptides and amino acids, the reader

is referred to the excellent presentations of Neurath and Schwert[123], Martin and Synge[111], Bamann and Myrbäck[11], and Popper and Necheles.[130].

Physiology. Each proteolytic enzymatic activity within the body operates as a complex system, as well exemplified by pancreatic trypsin. The first light thrown on the whole question came from the discovery that pancreatic juice collected without contact with the intestine does not digest protein. Early workers in Pavlov's laboratory[138] found an inert pancreatic factor, termed trypsinogen, which could be endowed with proteolytic activity by the addition of an extract of intestinal mucosa. The activating substance was called enterokinase. Guillaumie[72] noted that the rate of activation of trypsinogen was proportional to the amount of enterokinase added, supporting the view that the pancreatic proteolytic action was enzymatic in nature. Finally, the isolation in crystalline form of trypsinogen and of two other pancreatic proteases, chymotrypsinogen (1935) and procarboxypeptidase (1935), brought a clearer understanding. The long standing theory of Waldschmidt-Leitz, who regarded the activation of trypsinogen as stoichiometric combination of trypsin and enterokinase, was disproved. Conversion of the three precursors of pancreatic proteases: trypsinogen, chymotrypsinogen and procarboxypeptidase can be activated by trypsin. The conversion of trypsinogen to trypsin has been clearly demonstrated to be catalytic, since the identical crystalline trypsin may be isolated whether the trypsinogen activation is secured by the influence of enterokinase, by small amounts of added trypsin[99], or by a mold kinase (Penicillium kinase)[93].

Crystallized trypsinogen if dissolved in neutral solution, forms some trypsin immediately. This trace of trypsin then catalyzes the change further, resulting in an autocatalytic conversion. On the other hand, amorphous preparations of trypsinogen may contain trypsin inhibitor compounds which prevent its change to the active form. Activation is then rendered possible only by the addition of enterokinase. More recent studies to define further certain factors involved in the formation of trypsin, have shown that the autocatalytic conversion of trypsin from trypsinogen is markedly influenced by inorganic ions, being greatly accelerated by calcium or other alkaline earth salts. The rate of this autocatalytic process is maximal at pH 7 to 8 but the reaction is affected by the appearance of inert protein.

The enterokinase of the intestine, purified by Kunitz[94], has not yet been isolated in crystalline form. Its optimal enzymatic activity occurs at hydrogen ion concentrations ranging from pH 6 to 9. From extensive kinetic studies carried by Kunitz[95], the nature of the catalytic action of trypsin could be deduced more accurately.

Some Stimulants and Depressants of Pancreatic Proteolytic Function. The inhibiting effect of distention of the stomach on the secretory function of the pancreas was investigated by Bizard and Boulet[15]. These authors secured evidence suggesting that a diminution or even a complete inhibition of pancreatic secretion was called forth by the physical effect of distention. The possibility that this inhibition is mediated through the splanchnic nerves has been proposed. Favoring such a view is the marked similarity of this response to that of the biliary secretion under the same stimulation.

Chardon and Gross[29] found that an atmosphere poor in oxygen will reduce the volume of pancreatic secretion without alteration of its composi-

tion in anesthetized dogs receiving continuous intravenous injection of secretin. Pancreatic function returns to normal soon after supplying a proper concentration of oxygen. These investigators did not establish the critical level of oxygen saturation at which these changes take place.

The question may be raised whether cyanosis at birth or the presence of swallowed air in the stomach are of physiologic significance in inhibiting pancreatic activity at certain times in the early months of life.

It is of clinical interest that morphine acts indirectly as a depressant of the external secretion of the pancreas[100]. There is some indication of pancreatic stasis as reflected by a simultaneous rise of the pancreatic enzymes in the serum owing partly to contraction of the sphincter of Oddi. The production of the pancreatic amylase and trypsin is markedly decreased, several times as much as the depression in amount of bicarbonate. There is a sustained search for more selective agents capable of stimulating the secretory functions of the pancreas.

It is generally believed that the formation and flow of pancreatic secretions is under hormonal and nervous control. As a rule pancreatic response to a hormonal stimulant gives a copious flow of pancreatic juice with a poor enzymatic content. Conversely, the nervous stimulation mediated mainly through the vagal fibers is thick, scanty and rich in enzymes.

Secretin originating in the duodenum and upper jejunum, and pancreozyme obtained from extracts of duodenal mucosa, are responsible for the hormonal control. Both of these physiologic agents have been used as stimulants to evoke a good flow of pancreatic secretions during duodenal intubation for diagnostic tests[109,141]. The presence of secretin in the gastrointestinal tract of premature and newborn infants has been demonstrated by Baggenstoss and coworkers[10]. Combined tissue extracts of several postmortem specimens had to be used in order to obtain demonstrable quantities of this hormone. Apparently "secretin is present in the neonatal period but the amounts are probably so small that they are lost during the process of extraction when individual extracts are used"[10]. No studies have yet been reported on the pancreozyme in premature and newborn infants.

From the observations of Greengard and his coworkers[66,67], it is reasonable to conceive the mechanism of certain so-called sympathomimetic agents as being dissociated in their influence from the autonomic nervous system. Presumptive evidence is given by the enzyme-poor secretion elicited by a sympathomimetic drug, epinine. There is a similarity between the end-product of this stimulant and that of secretin. If we accept that secretin stimulation is essentially unaffected by atropine which paralyzes vagal endings, and that it is abolished by ergotamine which paralyzes sympathetic fibers, we are apt to conclude that secretin and epinine behave as though they have an autonomous action unrelated to the nervous system.

The effect of vagal stimulation on pancreatic secretion has also been reinvestigated by these authors[66,67]. Their findings allow general interpretations of interest. It appears that sodium nitrite, whose effect is mainly attributable to its action on the vascular bed, induces an enzyme-rich secretion similar to the one obtained with pilocarpine and prostigmine, acting through the vagus nerves. The same extensive response attained with sodium nitrite and parasympathomimetic drugs suggests that vagus excitation occurs partly through vasodilatation. An

increase in size of the pancreas at the time of vagus stimulation lends additional support to this view[6].

The relationship between pH of duodenal content and pancreatic secretions received the attention of Bayliss and Starling[12] as early as 1902. That the pancreas was responsive to acid introduced in the intestine was demonstrated by these workers. These experiments were critically appraised by Crider and Thomas[31] who found that acid as a stimulant for pancreatic secretion was effective when the pH was 4; a more copious flow was recorded when the pH was reduced to 3 or less. It is evident from the data submitted by the same investigators that two or more stimuli must be active at any time to promote an authentic pancreatic response. Corroborative evidence for this theory is given by the observation that the efficacy of pancreatic response is decreased when acid is introduced in the intestine after section of the vagus nerve.

The Phenomenon of Adaptation. In 1940, Dubos[41] showed that certain bacterial strains will develop the capacity to elaborate specific enzymes in response to predominance of a given substrate in the diet. A striking parallelism exists between this phenomenon in bacterial organisms and the adaptation of the pancreas to dietary composition. When rats are maintained for three weeks on a constant diet, an adaptation of the composition of the chief pancreatic enzymes to the predominant constituent of the diet is noted[70]. Thus a high carbohydrate diet elicits a pronounced increase in amylase output together with a decrease in trypsin. A high protein diet results in greatly increased trypsin output and a less extensive increase in lipase. On a high fat diet with a normal content of protein there are essentially no alterations in lipase or trypsin. A diet which is high in fat and low in protein causes a repression of all pancreatic enzyme formation, but addition of 1% choline to such a diet increases uniformly the content of all enzymes. These results are strictly reproducible only when these changes in the diet are prolonged.

To what extent this adaptation phenomenon is regulated by a humoral mechanism or by a reflex action was the next question to be answered. A working hypothesis was advanced by Grossman and his coworkers[71] which postulated that "the substitution of the hydrolyzed form of a foodstuff for the unhydrolyzed form will determine whether the presence of the undigested foodstuff in the gut is necessary for the elicitation of the characteristic pancreatic enzyme response." In the case of starch, the product of its enzymatic digestion, dextrose, is capable of exciting an increase in amylase. Conversely, casein hydrolysate does not induce an adjustment of the trypsin content such as is noted with casein. The authors postulate a nervous reflex factor in the case of trypsin regulation, and a humoral factor operating for amylase. Evidence is also presented which denies the intervention of pancreozyme, an extract of the small intestine which stimulates the secretion of digestive enzymes, influencing the volume rate of the pancreatic juice[75], in this type of adaptation[1]. Reactions initiated by pancreozyme are instances of immediate alteration in enzymatic composition of secretion, whereas the adaptation to diet in rats requires a prolonged period[2]. It is still necessary to explain how mechanisms known to increase pancreatic enzyme secretion cause a simultaneous alteration in all the chief enzymes, whereas adaptation to diet involves a selective increase in the enzyme acting on one predominant constituent of the diet.

Extent of Proteolytic Digestion. The proteolytic action of trypsin is different from that of pepsin in being carried on in an alkaline medium at pH 8. The preparatory digestion of protein by gastric pepsin is favorable to subsequent pancreatic tryptic activity. Though under physiologic conditions trypsin commences its action after the gastric juice has acted upon the dietary protein, it can also attack native protein such as meat or milk introduced directly into the duodenum. The final breakdown of hydrolyzed protein is assumed to be carried out by the succus entericus.

Crude trypsin has a weak milk-coagulating power before digestion occurs, provided the milk is not too slow in clotting[45,132,140]. Chymotrypsin has a powerful curdling action upon milk. Reference has already been made to the isolation of a crystalline fraction from lima beans exhibiting a powerful trypsin inhibitory action[153]. This property was investigated on a few proteinases known to clot milk[154]. The milk clotting power of pepsin or cathepsin was not inhibited whereas it was moderately so when chymotrypsin was the active agent, and most marked when trypsin was assayed.

The specific action of trypsin is to attack the proteose and peptone fragments of the protein molecule and break them into smaller units commonly termed peptides. In the earlier stages of tryptic digestion the amino groups are for the most part relatively large and are known as polypeptides. These are later split into groups containing 2, 3 or 4 amino acids, constituting the dipeptides, tripeptides and tetrapeptides. In the last stage of tryptic digestion free amino acids are released.

We have already referred to the specificity of trypsin activity in another section of this paper. We will now consider some of the biochemical mechanisms involved in the successive cleavage of proteins during the digestive process. The proteins and peptones are, without exception, subjected to an additional cleavage by pancreatic proteinases after a preceding exposure to gastric pepsin and hydrochloric acid[55]. Pancreatic proteinases break down casein to a greater extent than does pepsin-HCl alone.

According to modern concepts the peptide linkage -CH_2-CO-NH- is the predominant bond in the protein molecule. Fodor and his collaborators[55] have shown that partial cleavage by a pepsin-HCl mixture selectively breaks down pepsin-specific linkages. Further adding of pepsin and prolonging the time of digestion in the stomach might succeed in breaking down all these complexes. But since the time factor is brief[167], those linkages which are split by pepsin-HCl with difficulty but by pancreatic enzymes with ease are attacked by the latter enzyme.

As digestion proceeds, ultimately the peptonic mixture contained in the substrate yields tetrapeptides. These usually resist further splitting by proteinases but undergo cleavage by polypeptidases. When digestion has resulted in dipeptides, trypsin again attacks certain dipeptides. These consist of arginine and lysine compounds[123]. The following dipeptides have been shown refractory to hydrolysis by trypsin[126]: dl-leucyl-l-tyrosine, leucylglycylglycine, chloroacetyl-l-leucine, glycyl glycine, glycylaspartic acid and chloroacetyl-l-tyrosine.

Because of the important role of casein in the infant's and child's diet it becomes pertinent to add a few words on the digestion of casein by trypsin. This enzyme appears to digest casein so rapidly that coagulation is only apparent when low concentrations of the casein substrate are used or

when the reaction of the medium is so acid that proteolysis is necessarily slowed down. *In vitro* studies of one-hour digests of casein by crystalline trypsin[48] indicate that three fractions of increasing solubilities in water can be isolated. One fraction contains the greatest amount of cystine. Another fraction includes the highest concentrations of phosphorus, serine and threonine. A third fraction contains most of the free amino acids. Approximately half of the casein is converted during this time to a peptone-like substance. It is estimated that 80% of the cystine is liberated from casein digested with trypsin at pH 8 to 9[87].

To summarize some of the evidence gained by these studies, it appears that the enzymatic activity of pepsin and trypsin have some additive effects indicating that their individual action is dependent upon the configuration of amino acids adjacent to the peptide bonds. Pepsin has no known proteolytic property on synthetic polypeptides and hydrolyses only a negligible portion of native proteins to the stage of free amino acids. This implies that under physiologic conditions pepsin produces mainly a change in the colloidal aggregation of the protein in the direction of coagulation, whereas trypsin splits the molecular structure.

Growth Inhibitors. The problem of ascertaining minimum dietary protein requirements in humans has been complicated by the discovery of a nutrition inhibitor factor in soy beans by Ham and his collaborators[74]. These workers attempted to correlate their findings with the time-honored observation that heat treatment of soy bean improves its nutritive value. Accordingly the scope of investigations was enlarged and a systematic search of the biologic value of soy beans, legume seeds, lima beans and egg white fed to animals under various conditions of processing was initiated. In all these studies, attempts were made to correlate the action of antitrypsins with that of nutrition inhibiting activity. Three main approaches have been proposed to investigate this relationship. 1) The inactivation of antitrypsin by heat or the use of a competitive system where trypsin and antitrypsin are mixed in various proportions. 2) The addition of essential amino acids such as methionine to diets containing soy bean inhibitors. 3) The use of predigested diets providing a proteolyzed form of proteins, thus by-passing the factor of trypsin proteolysis. Here one is grateful for the careful studies of Liener and his coworkers[105] who reviewed the more significant contributions on this topic.

Preliminary observations that supplementing a soy bean protein diet with methionine increased its biologic effectiveness were substantiated by Melnik and his associates[115]. They attributed the impairment in utilizing protein derived from raw or improperly heated processed meals to an inhibition of the rate of methionine liberation rather than to a reduced degree of methionine availability. Further studies in the same line have shown that the slow rate of release of methionine was an inadequate explanation for the poor nutritive value of raw soy bean[78,133].

An interesting collection of data by Bowman[20,21], Ham[74] and Kunitz[98] correlated depressed nutrition with the presence of one or more antitryptic factors in unheated soy bean meal. The deleterious effect of this soy bean trypsin inhibitor on the growth of experimental animals was confirmed repeatedly by Ham[74], Almquist[2], Borchers[19] and others.

Liener and his coworkers[105] conducted extensive studies in an attempt to re-evaluate the effect of supplementary methionine on various diets con-

taining trypsin inhibitor. These authors postulated a two-fold mode of action regarding trypsin inhibitor preparations. "One which results in an impairment of the availability of methionine for growth as a consequence of its antiproteolytic activity and the other having an effect unrelated to the inhibition of proteolysis. The former can be demonstrated if the source of ingested nitrogen is an intact protein critically deficient in methionine. The latter effect is independent of the nature and quality of ingested nitrogen".

Desikachar[36] and Westfal[163] found that antitryptic preparations of raw soy beans have a growth depressing effect even when incorporated in diets containing papain-digested or otherwise well hydrolyzed proteins. They concluded that the trypsin inhibitor is not the factor responsible for the poor growth and utilization of intact protein, since the inhibitor of tryptic digestion affects unaltered dietary proteins only.

The isolation or purification of new trypsin inhibitors renewed the interest in the subject of animal growth inhibitors. In 1949, it was shown that a diet supplemented with crude lima beans displayed considerable growth inhibiting properties[153]. The trypsin inhibitor in egg white was prepared in purified form by Lineweaver and his associates[106] and assayed for its growth inhibiting activity by Klose[90]. Negative results were recorded with purified trypsin inhibitor from egg white, even when casein was used as a basic diet to eliminate the possibility of other protein constituents in raw egg white counteracting growth inhibiting activity. When seeds of 11 species of legumes were fed raw and autoclaved at a 12% level of dietary protein allowance, no discrepancy in nutritive value was observed[17]. Klose[90] came to the conclusion that since all growth inhibiting properties are derived from beans, the possibility remains that some trypsin inhibitors have growth-inhibiting activity for some reason other than inhibition of proteolysis.

In 1946, Kunitz[96] observed that crystalline soy bean antitrypsin and crystalline trypsin combine in definite proportions *in vitro*. Corroborative evidence for this finding was given by Almquist *et al.*[3] who studied the relation between the quantity of soy bean antitrypsin in the diet on growth rate of the chick. Growth inhibition is attained when the basic diet supplies raw soy bean at levels, not exceeding 5% of total dietary proteins, after which a plateau is reached. The authors assumed that the basic mechanism in this experiment was closely allied with a process of quantitative and specific interaction between the antitrypsin and the trypsin available in the chick.

In 1951, a new approach[18] based on the same principle of interaction of trypsin and antitrypsin has led to the important discovery that 5 parts of crude trypsin to 25 parts of raw soy bean will counteract the growth inhibiting effect of raw soy bean. Autoclaving this mixture for a prolonged period destroyed antitryptic activity but not growth-inhibiting activity. Further evidence offering support to these observations is suggested by the fact that no other untoward effects other than depressed growth have been reported as a result of feeding raw soy bean. Further inquiries along these lines should be profitable and might lead to some unsuspected clinical application in the field of pediatrics.

An interesting aspect of the role of tryptic activity in conditioning intestinal proteolysis has been inferred by Montgomery and his collaborators[117]. Fatty liver develops in insulin-treated depancreatized dogs. It can be prevent-

ed if these dogs are fed extra choline or extra methionine in free form. However lean meat or casein supplying adequate amount of bound choline and methionine fail to prevent fatty liver in these dogs. But when trypsin is added to such a diet, proteins are made available for lipotropic purposes, thus protecting the histochemical structure of the liver. In this sense, it is conceivable that trypsin indirectly possesses a lipocaic function and can be regarded as an anti-fatty liver factor[117].

Pharmacology of Trypsin Given Parenterally. This discussion embraces the knowledge of the biologic properties of trypsin beyond its role in digestive processes. Although the observations and conclusions presented here are derived from the views of many investigators, no attempt will be made to establish the antecedents of each viewpoint, but rather to bring more recent approaches to the appraisal of the reader.

Reactions of Tissues. A pharmacologic principle termed *bradykinin*[133c] is released coincidentally by trypsin, snake venom and kallikrein (see below). One might inquire into the mechanism of production and nature of this substance. When crystalline trypsin is incubated with the globulin fraction obtained by precipitation of plasma, a smooth-muscle stimulating substance of low molecular weight is released. In a similar way, when mammalian serum is mixed with certain snake poisons a substance is produced from the same globulin fraction which is able to cause constriction of the smooth muscle[161]. Furthermore, a thermolabile principle obtained by German workers[160] from pancreas and also from urine, termed kallikrein, liberates a substance resembling bradykinin when incubated with plasma or serum, but is without any releasing potency when acting on purified globulin.

The separate nature of trypsin and kallikrein is indicated by the finding that the soy bean trypsin inhibitor completely stops this pharmacologic property of trypsin but has no effect on kallikrein[133c]. This releasing property is not a common attribute of proteolytic enzymes, however, since chymotrypsin is incapable of liberating bradykinin from its precursor bradykinogen.

To complicate the problem, it appears that under certain circumstances trypsin alone has an action similar to that of kallikrein. The introduction of trypsin into the saline bath in which a strip of muscular tissue is suspended provokes a marked contraction. Inhibition is attained by the addition of arginine or histidine to the bath[39]. However, repeated exposure to trypsin results in an apparent desensitization phenomenon associated with the disappearance of the myofibril contractions. This last finding suggests that the basic mechanisms of parenteral tryptic activity may be closely allied with those of some anaphylactic processes. That this working hypothesis is not unreasonable is suggested by a variety of observations which may be summarized briefly as follows: 1) histamine is released when smooth muscle is perfused with trypsin solution[150]. 2) Intracutaneous injection of trypsin in heparinized rabbits is associated with a transit of histamine from cells to plasma[39]. 3) The intracutaneous injection of a small amount of trypsin leads to the localization of trypan blue at the injected site when the latter is injected intravenously. With increased amounts of trypsin, a local necrosis comparable to that of the Arthus phenomenon is produced[39]. 4) Subcutaneous injections fail to produce any visible or palpable tissue alterations if

crude or crystalline trypsin is first inactivated by heat or crystalline bovine serum albumin[139]. 5) Local necrosis produced by subcutaneous injection of trypsin is inversely proportional to the antiproteolytic activity of the serum[69]. An attempt to correlate further anaphylaxis and the pharmacologic effects of parenteral trypsin will be presented when reviewing tryptic activity on blood pressure and blood coagulation.

Reactions of Blood Pressure and Coagulation Mechanisms. One would expect enzymatic inactivation of pepsin injected intravenously because the pH of the blood is not favorable to its action. Trypsin, however, evokes a diversity of dramatic changes when injected intravenously. The two most prominent features are *a*) a hypotensive action and *b*) a simultaneous change in the blood coagulation system. Both effects are reflected as a state of shock.

If the trypsin is introduced rapidly in experimental animals, there occurs within 15 to 25 seconds a fall in systemic blood pressure with little or no change in the pulmonary artery pressure[150]. There is congestion of the liver and viscera with rise in the portal vein pressure. Blood pressure returns to normal within 6 to 16 minutes while a shock-like state persists during the same length of time. If however, the trypsin is injected slowly, these effects are inhibited by the natural antitryptic properties of the serum[63].

Curiously enough, bradykinin initiates a fall in blood pressure similar to that caused by trypsin. Rocha e Silva[133d] demonstrated that the hypotension resulting from bradykinin is long-acting, whereas that of trypsin is sudden and short-acting. He inferred that in tryptic shock the initial fall in blood pressure is due to trypsin. After a few minutes there is a slight increase in the blood pressure followed by a more sustained fall lasting 10 to 15 minutes and due to bradykinin.

Biochemical analysis of the pharmacologic action of trypsin in shock demonstrates that free amino or phenolic groups do not appear to affect the hypotensive activity of trypsin[22]. Characteristically, free tryptophane participates in this phenomenon. Iodination serves to decrease greatly the hypotensive influence of intravenously administered trypsin with a minimum of alteration of its proteolytic activity.

The changes in blood coagulation mechanism present a paradoxical picture. If one assumes that trypsin acts as a catalytic agent in the conversion of profibrinolysin to fibrinolysin (fibrinolysokinase)[84] and in the activation of prothrombin to thrombin (thrombokinase)[51], an antagonism between coagulation and anticoagulation would be expected. From experimental data gathered by two groups of workers, it appears that both effects can occur with results depending on the rate of infusion of crystalline trypsin, the enzymatic concentration and the total dose.

If a small dose of crystalline trypsin is introduced rapidly into the veins, a massive macroscopic intravenous coagulation is described in the majority of animals[39,133a]. Coincident with these macroscopic changes, there is a prolongation of the clotting time and an increase of the prothrombin time[39]. Furthermore, the histamine content of the blood is raised to a point proportional to a decreased histamine content of the liver.

We are indebted to Innerfield and his colleagues[80,81,82] for their carefully controlled re-evaluation of the effect of intravenously administered trypsin upon the protein components of the coagulation mechanism. When large doses of a solution of crystalline trypsin are infused at a rate of 35 to 40

drops a minute, a point is reached beyond which all clotting proteins are proteolyzed. This is characterized by depressed levels of prothrombin, acglobulin, fibrinogen, antifibrinolysin and antithrombin. These workers emphasize that shock and intravascular coagulation do not appear by their method. They suggest that this anticoagulant effect does not necessarily prove a direct trypsin-fibrin substrate interaction but more probably an activation of the fibrinolytic system. An important observation was that intravenous trypsin had a lytic effect on experimentally induced thrombin in the femoral vein of dogs.

In vitro, it is demonstrated that in appropriate amounts trypsin digests fibrin and fibrinogen, thrombin and prothrombin[44,76]. Both crude and crystalline trypsin are shown to clot oxalated plasma, more so when potentiated by calcium ions[52,54]. These latter observations are supported by the evidence that trypsin inhibitor from soy bean retards the coagulation of normal blood, recalcified plasma and recalcified plasma to which an excess of thromboplastin (thrombokinase) is added[32,151]. The soy bean inhibits the coagulating activity of crystalline trypsin on oxalated plasma. It does not exert any antithrombic action as evidenced by failure to interfere with the coagulating property of thrombin on oxalated plasma or purified fibrinogen.

At this point it is appropriate to review our present knowledge on the alterations of the coagulability of the blood, with special reference to anaphylactic shock derived from the intravenous injection of peptone, thrombin shock caused by intravenous administration of thrombin, and trypsin shock discussed above[133b]. In these three types of shock the hypotension and collapse syndrome appear to be identical. In all the clotting time is prolonged.

When peptone is injected intravenously into sensitized animals a series of highly significant reactions occur after a few seconds[150]. There is a period of increased coagulability of the blood followed by incoagulability; histamine and heparin are released, and there is markedly prolonged clotting time of the recalcified plasma. Although the true anaphylactic phenomenon involves the interaction of antigens and antibodies, an anaphylactic-like reaction can also be produced without the participation of an antigen-antibody reaction.

A conspicuous discrepancy between anaphylactic and trypsin shocks is the release of heparin in the former mechanism, without any changes in the prothrombin time and fibrinogen content of the blood. Furthermore, the pale non-collapsed lungs indicate that death is due to asphyxia resulting from bronchostenosis.

In shock produced by thrombin, the predominant changes consist of a decreased content of fibrinogen and an increased prothrombin time in the plasma, reflected in a prolongation of the clotting time[150]. Intravascular coagulation becomes macroscopically demonstrable. This is parallel to what occurs in trypsin shock. However, preliminary heparinization of experimental animals does not abolish the vasodepressive action of trypsin, though it interferes with that of thrombin. This indicates that the vasodepressive action of thrombin is chiefly correlated with its clotting ability[150] while that of trypsin is related to some other mechanism. Nevertheless heparinization in animals does exert some protective action against doses of trypsin that would otherwise be lethal.

Action of Trypsin on Hemagglutinins *in Vitro*. It has long been known

that agglutination of erythrocytes *in vitro* is a standard method for detecting anti-red cell serum antibodies. The effect of trypsin on hemagglutinating systems has been the object of much attention since the pioneer work of Morton and Pickles in 1947[120]. Crystalline trypsin solution added to a suspension of washed, heparinized red cells incubated for varying lengths of time alter these cells. The procedure has been found[120] to facilitate agglutination of red cells sensitized with incomplete antibody and also to enhance the specific agglutination by other hemagglutinins. The incomplete antibody, also termed univalent or soluble antibody, will not precipitate directly with the antigen unless certain adjuvants are present[42]. Nonprecipitating incomplete antibodies have been found in sensitization states to Rh and other minor blood group antigens, and in acquired hemolytic anemia due to autogenous antibodies. Trypsin-modified red cells are more likely to give positive results with weakly reacting antibodies[120].

Substantially higher titers have been observed with auto-antibodies of acquired hemolytic anemia which were detectable in low titer by the Coombs technique[164]. There may also be slightly greater agglutinability of trypsin-treated cells in anti-A, anti-B, anti-M and anti-N sera as compared to untreated cells[164]. Trypsin-modified cells show no increased ability to detect the currently known antibodies against the antigens Lewis, Lutheran and S[120].

The exact mechanism of action of trypsin on red cells is still obscure. Trypsinized cells absorb somewhat more antibody from serum than do normal cells and therefore, as test cells, are more sensitive in the detection of incomplete antibodies even with salt solution as a diluent[134]. An adjunct to this property is the absence of prozone and the readability when trypsin-modified red cells are used. When serial studies are performed on patients, alterations of titers are demonstrated with greater ease by the use of trypsin. The specificity related to the agglutination with trypsin has been questioned by Rosenthal[135] who described an agglutinin present in most normal sera capable of agglutinating trypsin-modified erythrocytes.

As evidenced by the work of Ponder[129] human red cells treated with trypsin undergo certain changes of their physical properties. Their volume is slightly increased, their osmotic and mechanical fragilities are augmented and their electrophoretic mobility is reduced.

Certain new biologic properties[164] characterize the trypsin-modified red cells. Crystalline antitrypsin added to cells after tryptic treatment does not alter their newly acquired properties. Immunization of rabbits with trypsin-modified red cells leads to the production of antibodies which are not completely absorbable by untreated cells. It seems likely that this proteolytic enzyme actually modifies the surface of red cells so as to rearrange the antigenic moiety of the cell.

Summary of Theoretical Data. During the last decade, more standardized methods for determination of trypsin activity and the isolation of a number of trypsin inhibitors in crystalline form have contributed to a better understanding of some pharmacologic properties of this proteolytic enzyme. During the same period, considerable attention has been given to some basic problems which may have as yet unrecognized clinical significance in the field of pediatrics:

1) It is suspected from the pancreatic secretory response to certain stimulants and depressants that anoxia of the newborn and distention of the

stomach during infancy might exert an inhibitory action on pancreatic function and trypsin output.

2) It has been demonstrated that animals and infants maintained on a constant diet will develop a phenomenon of self defense consisting in the adaptation of the composition of pancreatic enzymes to the predominant constituents of the diet. A high protein diet results in increased trypsin output and a less extensive but definite increase in lipase.

3) The enzymatic action of trypsin is narrow, being specifically dependent upon the configuration of amino acids within the protein molecule. Casein is readily digested by trypsin, which explains its rather poor ability to coagulate milk. During the process of digestion under physiologic conditions of buffer and time factors, pepsin produces mainly changes in the colloidal aggregation of the protein substrate, whereas trypsin splits the molecule structure down to the stage of polypeptides and free amino acids.

4) Certain growth inhibiting factors may play an important part in the child's diet as evidenced by investigations on antitryptic factors isolated from raw beans and other raw foods.

5) Rapid intravenous injection of trypsin brings a sudden state of shock, a fall in the blood pressure analogous to anaphylactic shock, and immediate coagulation of the blood. If given by slow infusion, trypsin may affect the clotting mechanism in the direction of anticoagulation.

6) Trypsin-treated erythrocytes usually absorb somewhat more antibody from serum and facilitate serologic tests for the incomplete antibodies found in erythroblastosis fetalis and acquired hemolytic anemia due to auto antibodies.

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BOOK REVIEWS AND NOTICES

THE CIBA COLLECTION OF MEDICAL ILLUSTRATIONS. VOLUME 1: NERVOUS SYSTEM. BY FRANK H. NETTER, M.D. Pp. 143; 104 ills. in full color. Summit, N. J.: Ciba Pharmaceutical Products, Inc., 1953. Price, $6.00.

THE accuracy, attractiveness and educational worth of Dr. Netter's plates are well known to most physicians through previous distribution of several kinds. This volume provides (at cost) in book form a complete and organized compilation of his plates on the nervous system. (Similar volumes on the other body systems are promised for the future.) Descriptive text for each of the 64 plates covering the anatomy of the spinal cord, the central nervous system, and the pathology of the brain and spinal cord is written by Dr. Abraham Kaplan; that for each group of 20 plates on functional neuroanatomy and the autonomic nervous system, respectively, is by Dr. Gerhardt von Bonin and Dr. Albert Kuntz.

This is an atlas-text which can be recommended to every entering medical student for it will be useful throughout his medical curriculum; and later in his practice. Notation must be made, however, that on page 37 the text is obviously wrong in stating the course of the posterior spinal artery as "downward in the midline of the dorsal surface of the spinal cord"; its correct course is beautifully illustrated and described on page 53 (Plate 30). The only other inconsistency noted by the reviewer is the different innervation of the lachrymal gland as shown on Plate 20 from that on Plate 54. The representation on the latter plate will probably be more generally deemed correct. Except for these points, Dr. Netter's illustrations and the textual material alike provides a concise yet comprehensive coverage of a complex and oftentimes controversial subject. N. B.

DERMATOLOGIC FORMULARY. Edited by FRANCES PASCHER, M.D., New York Skin and Cancer Unit of the New York University Hospital and the Department of Dermatology and Syphilology of the New York University Post-Graduate Medical School. Revised, 1953. Pp. 150. New York: Paul B. Hoeber, Inc., 1953. Price, $3.00.

IF ONE assumes the need for a dermatologic or other formulary for instructional or economic reasons, then Pascher's Dermatologic Formulary is probably the best compilation of this sort the reviewer has seen. In a broad way, this small volume covers the essential topical remedies, systemic therapy, and articles for clinic use. In addition to giving adequate detail on the various preparations, a few words on the philosophy of the particular modality described and a comprehensive index complete the volume.

A formulary is a rather personal matter. The experience of the author or authors dictates what should be included. This may account for the absence of P.P.D. (tuberculin). Furthermore, a formulary should not be static. Coming from the institution where Compound F (hydrocortisone) ointment is so highly regarded, the format of the present volume does not allow its inclusion until a completely new edition is made. If the book were of a loose-leaf type and on better paper (to withstand constant use by those for whom it is intended) insertions could be made readily. This type of setup would broaden the usefulness of the formulary; by the addition of certain "pet" remedies, it could thus serve as the official list for any dermatologic institution. This is as it should be since there is more to this Dermatologic Formulary than meets the eye. H. B.

METHOD OF ANATOMY. BY J. C. BOILEAU GRANT, M.C., M.B., Professor of Anatomy, University of Toronto. 5th ed. Pp. 870; 862 ills. Baltimore: Williams & Wilkins Co., 1952. Price, $7.00.

THERE are 18 more pages in this new edition than in the previous one. This increase in the number of pages is due to the addition of 78 new illustrations and does not represent an increase in the volume of text material. There have been many minor changes in the written material for the purpose of clarification and correction of small errors. Sixty-nine of the old illustrations have been redone either completely or in part and the new versions are definitely more pleasing to the eye and achieve a good impression of depth. This continues to be an excellent textbook presenting many basic concepts of anatomy and achieving a logical organization of material along regional lines. J. H.

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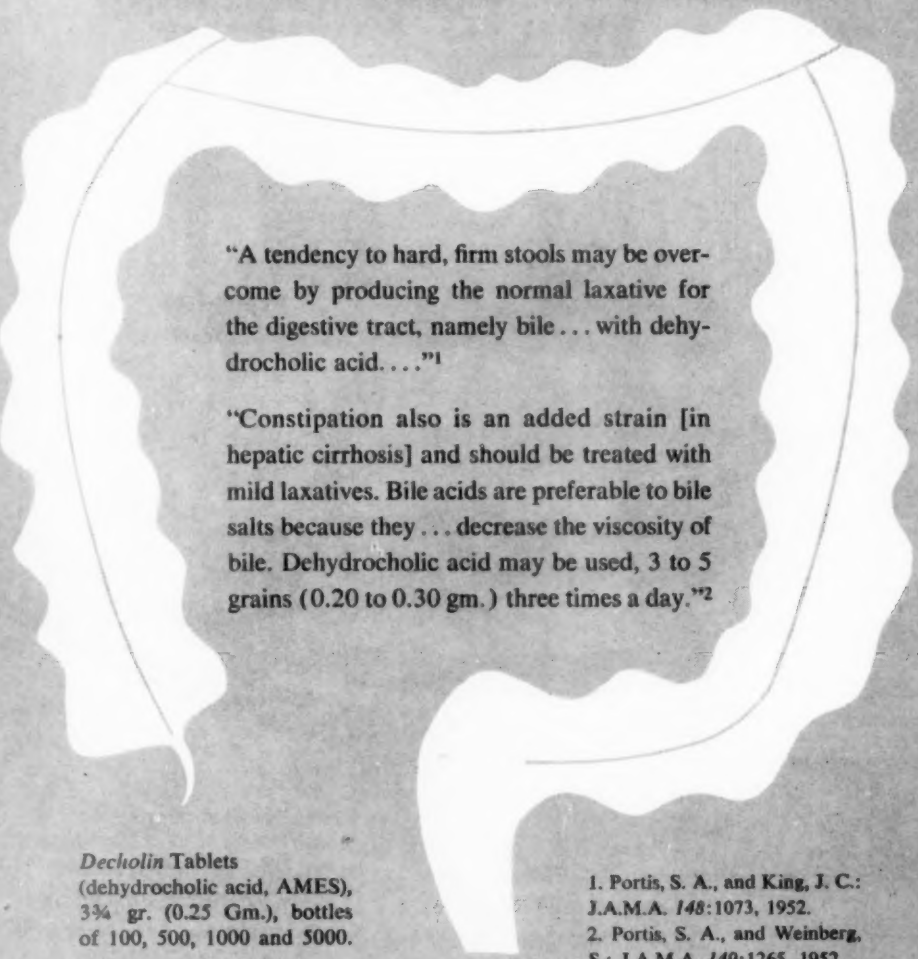

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2. Portis, S. A., and Weinberg, S.: J.A.M.A. *149*:1265, 1952.

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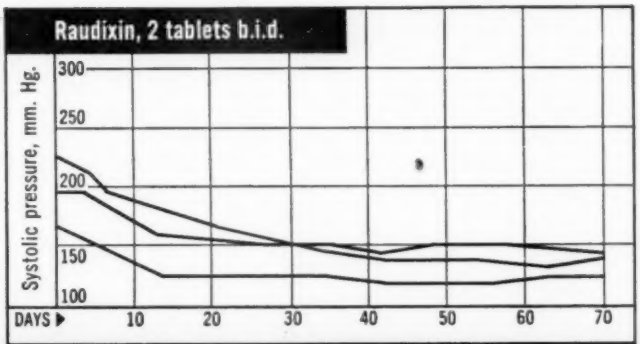

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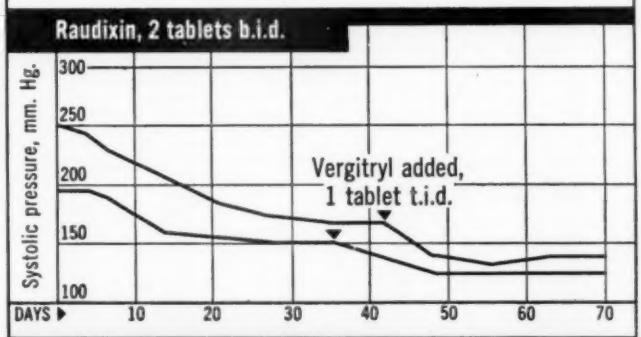

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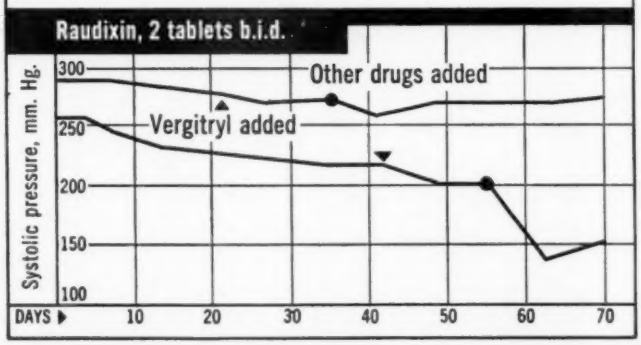

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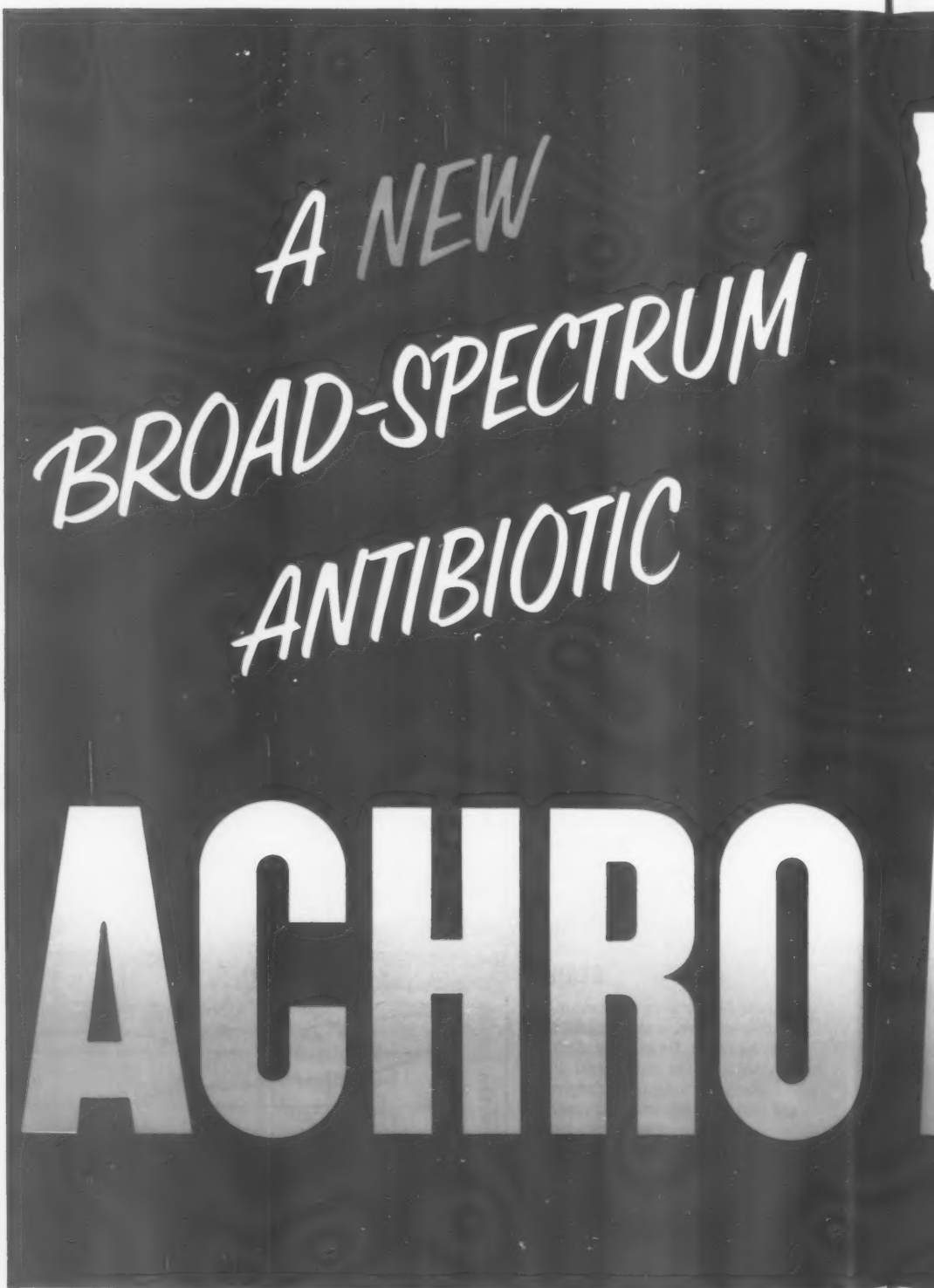
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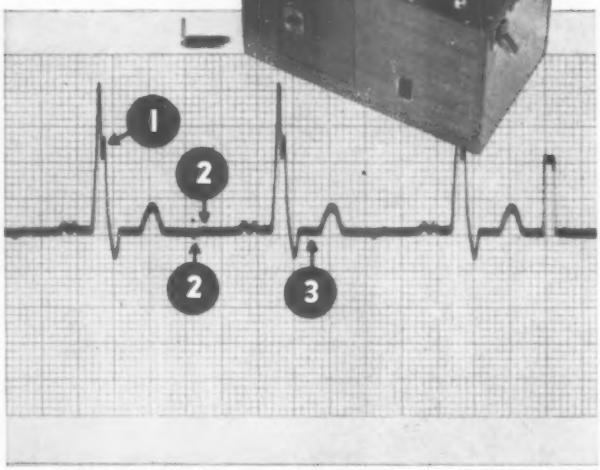

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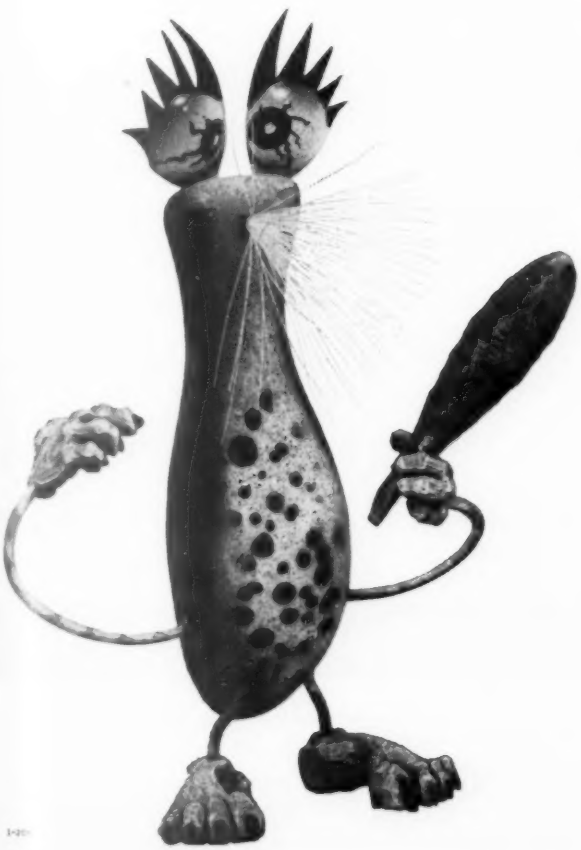

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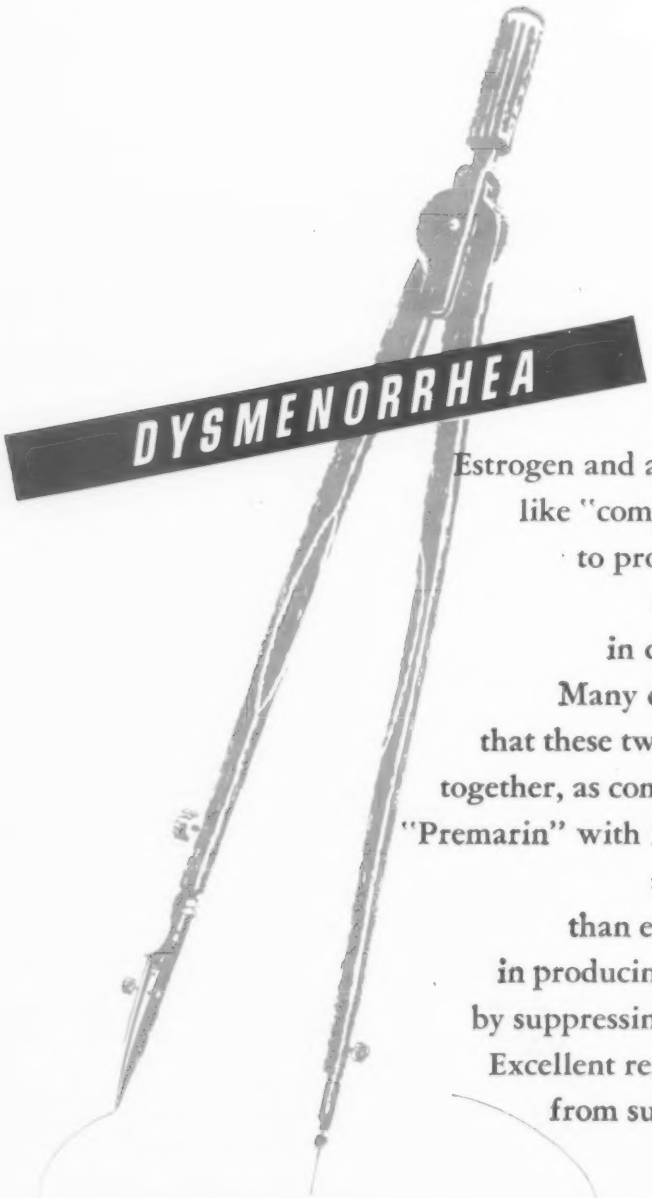

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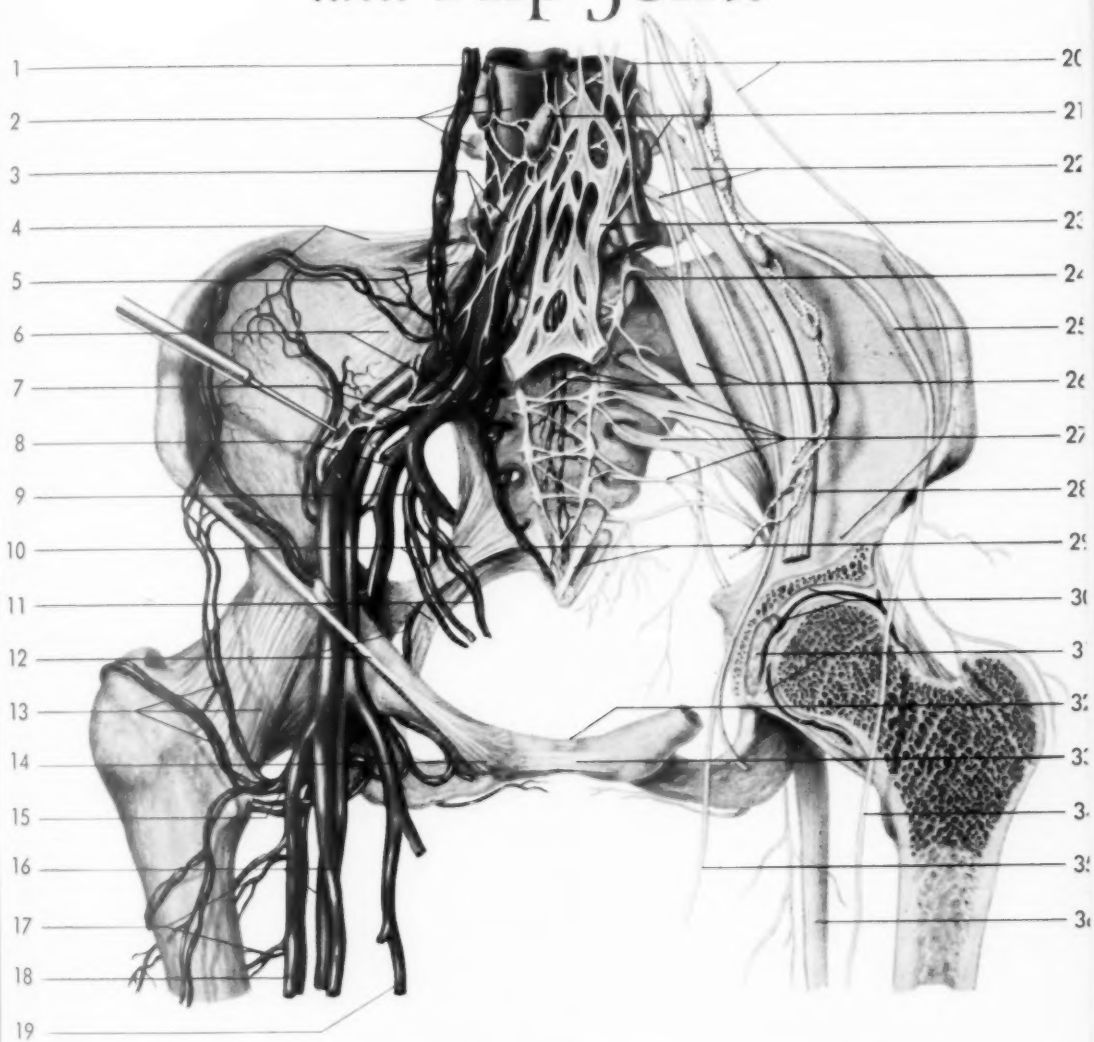

1 Ovarian artery and vein
2 Vena cava; lumbar lymph nodes
3 Right common iliac artery and vein
4 Iliolumbar ligament; branches of iliolumbar artery and vein
5 Lumbosacral ligament; superior gluteal artery and vein
6 Anterior sacroiliac ligament; internal iliac (hypogastric) artery
7 External iliac artery and vein
8 Obturator artery and vein
9 Inferior gluteal artery and vein
10 Sacrospinous ligament; uterine artery and vein
11 Sacrotuberous ligament; vaginal artery and vein
12 Inguinal ligament; internal pudendal artery
13 Iliofemoral ligament; branches of lateral femoral circumflex artery and vein
14 Lacunar ligament
15 Lateral femoral circumflex artery and vein
16 Femoral artery and vein
17 Perforating arteries and veins
18 Deep femoral artery and vein
19 Great saphenous vein
20 Aorta; ilioinguinal nerve
21 Lateral aortic lymph nodes
22 Lumbar nerves
23 Hypogastric sympathetic plexus
24 Sympathetic trunk
25 Lateral femoral cutaneous nerve
26 Middle sacral artery and vein; lumbosacral trunk
27 Sacral nerves
28 Femoral nerve
29 Lateral sacral artery and vein; anterior sacrococcygeal ligamen
30 Lunate articular cartilage; joint cavity
31 Acetabular fat pad; ligamentum teres
32 Interpubic fibrocartilage
33 Superior pubic ligament
34 Anterior branch of lateral femoral cutaneous nerve
35 Obturator nerve
36 Great sciatic nerve

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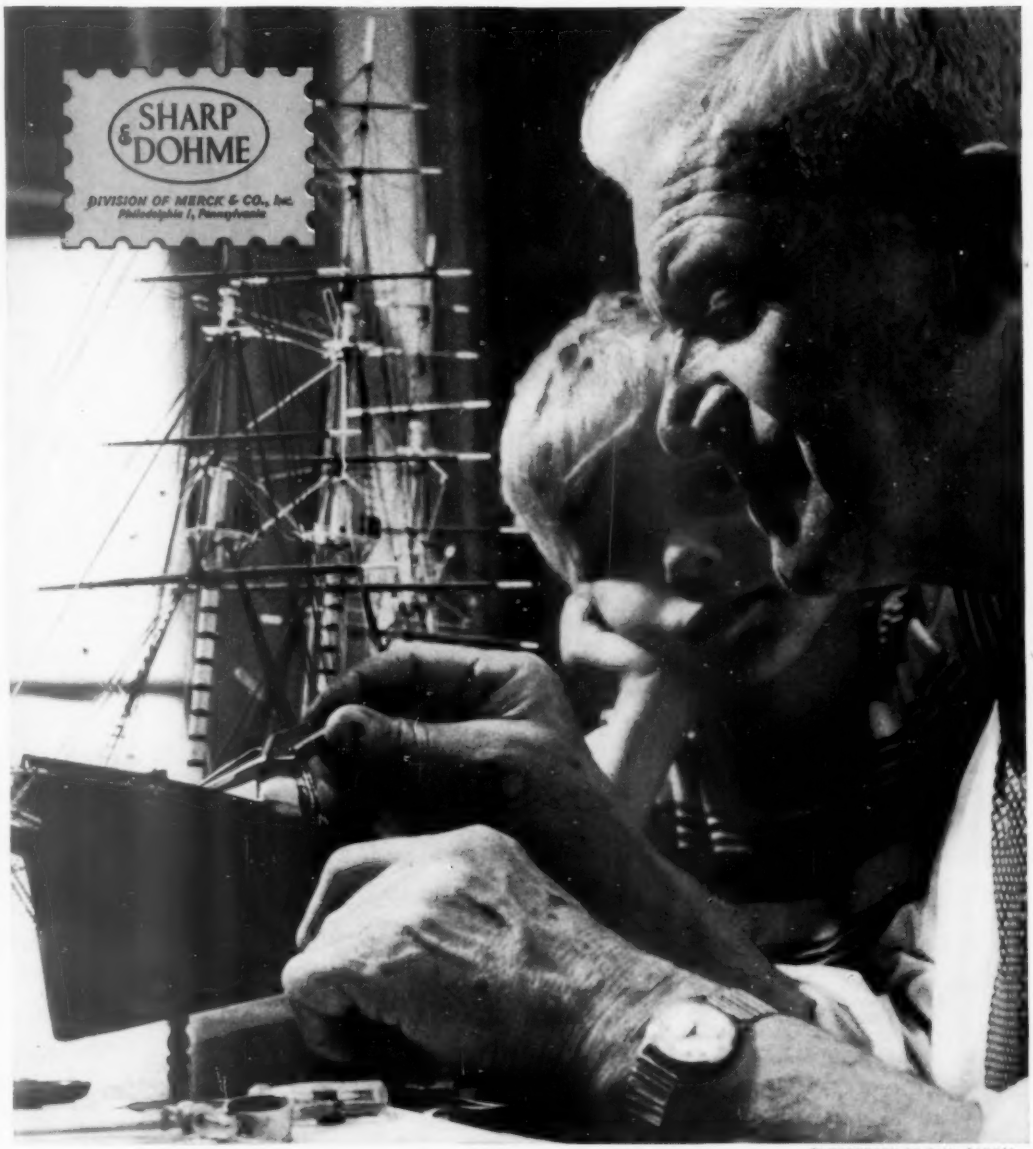

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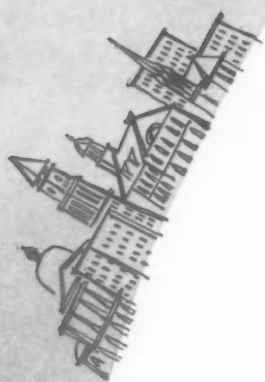

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1. Rogers, M. P., and Gray, C. L.: *Am. J. Digest. Dis.* 19:180, 1952.

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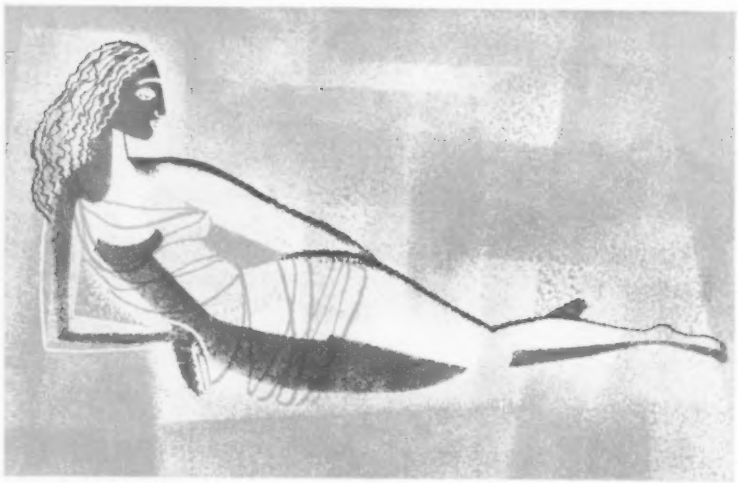

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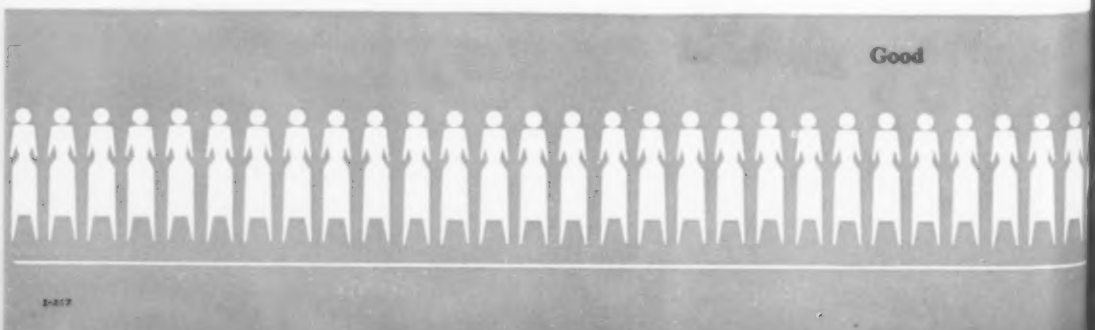

IN CLINICAL TESTS, Lathrop and Carlisle[1] treated 63 women for hypermenorrhea, using the new BLUTENE therapy. Dosage was oral, at home. Each patient took one 100 mg. tablet of BLUTENE after meals, 3 times daily, for the duration of bleeding.

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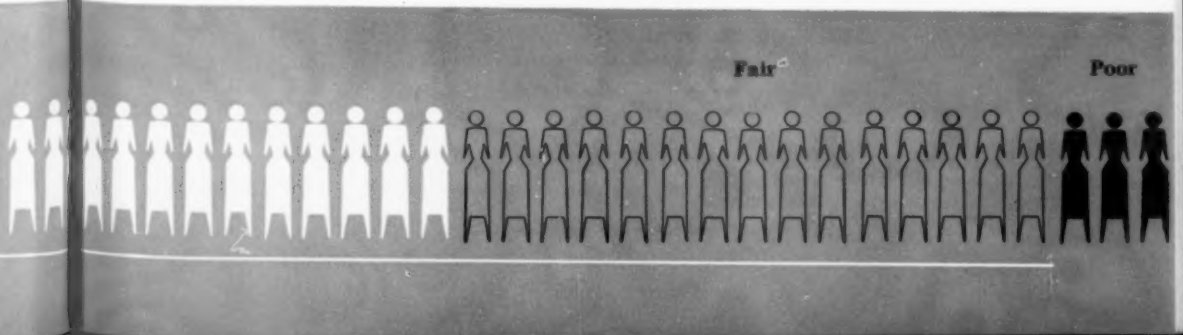
1. Lathrop, C. A., and Carlisle, W. T., Oral Toluidine Blue in the Treatment of Hypermenorrhea, Amer. J. Obst. & Gynec., 64:1376, December, 1952. 2. Rumbolz, W. L., Moon, C. F., and Novelli, J. C. Use of Protamine Sulfate and Toluidine Blue for Abnormal Uterine Bleeding, Amer. J. Obst. & Gynec., 63:1029, May, 1952. 3. Priddle, Harold, Discussion of Rumbolz et al., Amer. J. Obst. & Gynec., 63:1036, May, 1952. 4. Bickers, W., Toluidine Blue—An Evaluation in the Treatment of Uterine Bleeding, in press, Amer. J. Obst. & Gynec.



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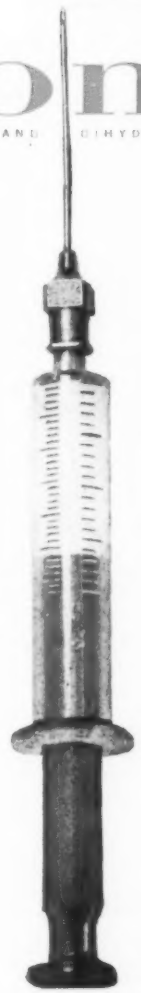

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1. Heck, W. E.: Reduced Ototoxicity by Combined Streptomycin-Dihydrostreptomycin Treatment of Tuberculosis, Scientific Exhibit 317, 102nd Annual Meeting A.M.A., New York, June 1-5, 1953.

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**Annals of Internal Medicine*, 37:465, 1952.

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CLINICAL DISORDERS of the HEART BEAT

By SAMUEL BELLET, M.D.

Director, Division of Cardiology, Philadelphia General Hospital; Director, Division of Cardiovascular Diseases, Graduate Hospital, University of Pennsylvania; Associate Professor of Cardiology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, etc.

ESSENTIAL features which have an immediate relation to clinical disorders of the heart beat are considered in detail in this helpful book. It is essentially *a guide to diagnosis and treatment*, and is based on the author's long personal experience with cardiac patients in the wards of the Philadelphia General Hospital.

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(Published October, 1953)

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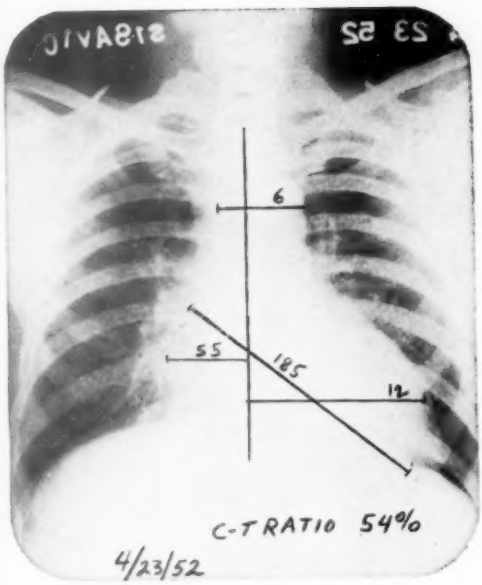
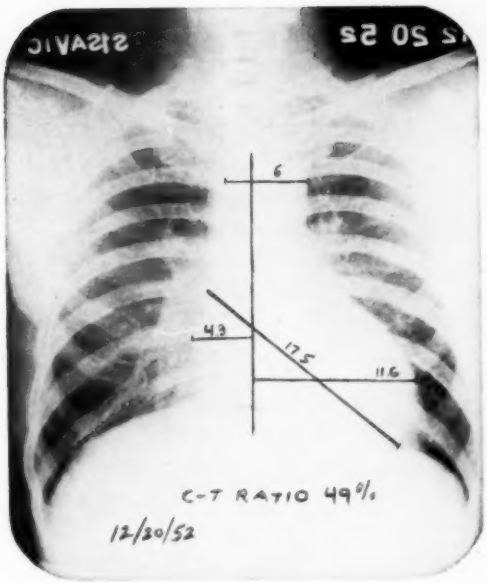


Patient F.S. before Methium: Cardio-thoracic ratio 54%, blood pressure 240/160 mm. Hg.[1]



After Methium: Cardio-thoracic ratio 49%, blood pressure 160/100 mm. Hg. This patient (F.S.) experienced no toxic side effects and did not lose a single day of work.[1]

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